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EFFECT OF LIPID ON
THE FORMATION OF VOLATILE AROMA COMPOUNDS
BY THE MAILLARD REACTION

by

Linda J. Farmer

a Thesis submitted to the University of Bristol

for the Degree of Doctor of Philosophy

Institute of Food Research-
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August 1990

MEMORANDUM

Except as acknowledged elsewhere, the work presented in this dissertation is that of the author alone. The material has not previously been presented to the University of Bristol, or any other establishment, for a higher degree.

Signed, Linda Farmer

Date 31/8/90

ACKNOWLEDGEMENTS

I am especially grateful to Dr D.S. Mottram and Dr R.L.S. Patterson who have given generously of their advice and encouragement throughout the course of this project, despite the difficult times.

In addition, I wish to thank Dr F.B. Whitfield, of CSIRO, Australia, and my colleagues in the Food Chemistry Section for their continued interest and moral support. I am also indebted to Ms S. Brock, Mr M. Lewis, Mr A. Watson and Miss S. Wong for the operation of the gas chromatograph-mass spectrometers and gas chromatograph-infrared spectrophotometer.

I thank Professor A.J. Bailey and the Institute of Food Research for permitting me to submit the results of my research for the degree of Doctor of Philosophy and I acknowledge the financial support provided by the Ministry of Agriculture, Fisheries and Food for the Flavour Group at IFR-Bristol.

ABSTRACT

The Maillard reaction between amino acids and sugars and the thermal degradation reactions of lipids both yield a wide variety of volatile products of importance to the flavour and aroma of cooked foods. However, the role of interactions between these reactions in the generation of desirable flavours has received little attention. Previous work has suggested that interactions between phospholipids and the Maillard reaction may play a key role in the generation of the aroma of cooked meat. This Thesis describes the use of simple model systems, containing an amino acid, sugar and lipid, to study the effect of reactions between these components on the formation of volatile aroma compounds.

More than 200 volatile compounds have been identified in Maillard reactions between glycine or cysteine, ribose and phospholipid. These include a number of compounds not reported previously in model systems or in foods. Heterocyclic compounds predominate in the absence of lipid, while the inclusion of phospholipid not only adds aliphatic products of lipid oxidation, but also compounds specific to the interaction of lipid in the Maillard reaction. Phospholipid also increases the number and range of individual odours detected in the reaction mixtures and has a marked influence on the overall aroma of both Maillard systems; the aroma of the cysteine + ribose + phospholipid reaction mixture has a persistent meat-like note.

A comparison of the effect of four different lipids on the aroma volatiles from the cysteine + ribose Maillard reaction has demonstrated marked dissimilarities not only between the triglyceride and phospholipids, but also between the three phospholipids studied. In addition to the modification of the volatile products of the Maillard reaction by lipids, the presence of Maillard reactants also alters the amounts of aliphatic compounds derived from lipid degradation. The differences in behaviour between the various lipids appear to be due to dissimilarities in their fatty acid compositions and phospholipid polar moieties.

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CHAPTER 1

INTRODUCTION

1 INTRODUCTION

The flavours of cooked foods are formed as a result of reactions between flavour precursors which occur during heating. These precursors may be derived from lipid, carbohydrate or protein constituents of foods or from minor components such as vitamins, nucleotides etc. Although some flavour compounds may arise from specific biosynthetic pathways during the growth of the plant or animal, most of the volatile aroma compounds associated with cooked foods are formed during the cooking process by two main types of reaction: the Maillard reaction and the thermal degradation of lipid.

The Maillard reaction between reducing sugars and amino acids is responsible for the formation of many heterocyclic compounds with distinctive aromas and low odour thresholds. Similarly, the oxidation of the lipid constituents of food contributes many highly odorous aliphatic compounds causing both pleasant and undesirable odours. Both reactions have been studied widely and their principal mechanisms are discussed in Sections 1.1 and 1.2. Rather less information has been learned about the interactions which occur between these two reactions. This subject, of particular relevance to the work described in this Thesis, is reviewed in Section 1.3.

Section 1.4 presents the aims of the studies described in this Thesis in the context of the state of scientific knowledge outlined in the previous Sections.

1.1 THE MAILLARD REACTION

Ever since mankind learned to appreciate the advantages of cooking food, human beings have been familiar with some of the effects of the Maillard reaction. It would have been evident that cooking not only prevented spoilage of foods and changed their texture, but also conferred on many foods a brown colour and an altered or improved flavour. It is not unreasonable to assume that the aroma of cooking food would have been as appealing to our distant ancestors as it is to ourselves.

In more recent times, the Maillard, or non-enzymatic browning, reaction has become the subject of scientific as well as gustatory interest. Louis Maillard (1912) was the first to report the formation of brown-coloured products on heating glucose with glycine. Since then the Maillard reaction, as it has become known, has been the subject of numerous publications and many reviews. It is now known that this reaction not only affects the colour and flavour of cooked foods but also has nutritional consequences due to the depletion of essential amino acids and carbohydrates and that the products of the Maillard reaction may have mutagenic and antioxidant properties (Mauron 1981; Barnes *et al* 1983; Bailey *et al* 1987).

The Maillard reaction between reducing sugars (or other carbonyl compounds) and amino acids (or peptides, proteins etc), comprises a complex network of pathways, whose many products range from high molecular weight pigments (melanoidins) to the volatile, low molecular weight compounds contributing to aroma. The complexity of this reaction is illustrated by the reaction scheme suggested by Hodge (1953), which is still largely accepted today (Fig. 1.1A).

Of primary importance to the work described in this Thesis is the important role played by the Maillard reaction in the generation of compounds contributing to aroma; many of the compounds responsible for the characteristic odours of cooked foods are thought to be formed by this reaction. Other aspects of the Maillard reaction (effects on colour, nutritional and mutagenic

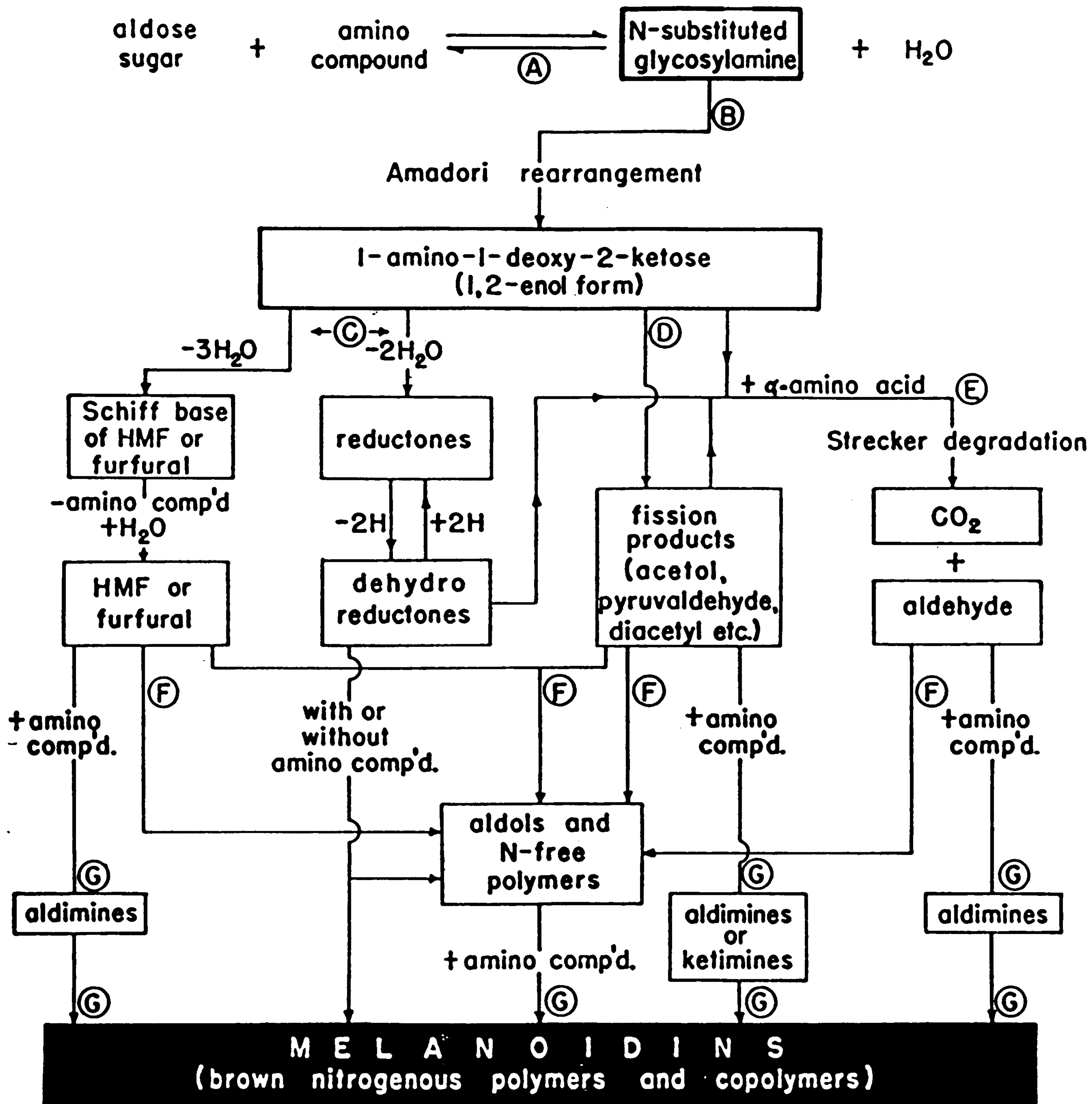


Figure 1.1A: Diagram from Hodge (1953) showing major Maillard pathways leading to melanoidin formation.

I Initial stage	A	Sugar-amine condensation
	B	Amadori rearrangement
II Intermediate stage	C	Sugar dehydration
	D	Sugar fragmentation
	E	Amino acid degradation
III Final stage	F	Aldol condensation
	G	Aldehyde-amine polymerisation; formation of heterocyclic nitrogen compounds

properties) are outside the scope of this work and will not be considered.

The formation of volatile compounds by the Maillard reaction has been the subject of a number of excellent reviews (Hodge 1953; Hodge 1967; Mauron 1981; Hurrell 1982; Vernin and Parkanyi 1982; Danehy 1986) and therefore, the review presented in this Thesis will be confined to a summary of the mechanisms of the initial stages of the Maillard reaction, and of some further reactions responsible for the formation of volatile products. Factors affecting the reaction will be discussed and the literature on glycine and cysteine-containing Maillard systems assessed.

1.1.1 MECHANISMS OF THE MAILLARD REACTION

The mechanisms of the Maillard reaction can be divided into three main stages. 'Early' Maillard reactions (steps A and B in Fig. 1.1A) include the condensation of sugars with amines to give N-substituted glycosylamines, and the rearrangement of these compounds into isomeric forms by the Amadori or Heyns rearrangements (Hodge 1953; Danehy 1986). 'Advanced' stages of the Maillard reaction (C, D, E in Fig. 1.1A) involve breakdown of Amadori and Heyns compounds by deamination, dehydration and fragmentation. Further interactions between the many products of these reactions can give a wide range of volatile compounds and also polymeric browning products.

While the initial stages of the Maillard reaction have been largely elucidated, many of the later pathways remain little understood. The reaction of one amino acid and one reducing sugar may yield several hundred products and the mechanisms of formation of many of these are the subject of continuing investigation in many laboratories.

1.1.1.1 'Early' Maillard reactions

Formation of N-glycosylamine; Amadori and Heyns rearrangements

The first step of the Maillard reaction involves the condensation of the carbonyl group of a reducing sugar (in the open chain form) with the primary amino group of an amino acid, peptide etc (Fig. 1.1B; Hodge 1953). The elimination of water gives a Schiff base, which cyclizes to give an N-substituted glycosylamine. This compound is of limited stability and is spontaneously converted, by an acid-catalysed reaction known as the Amadori rearrangement,

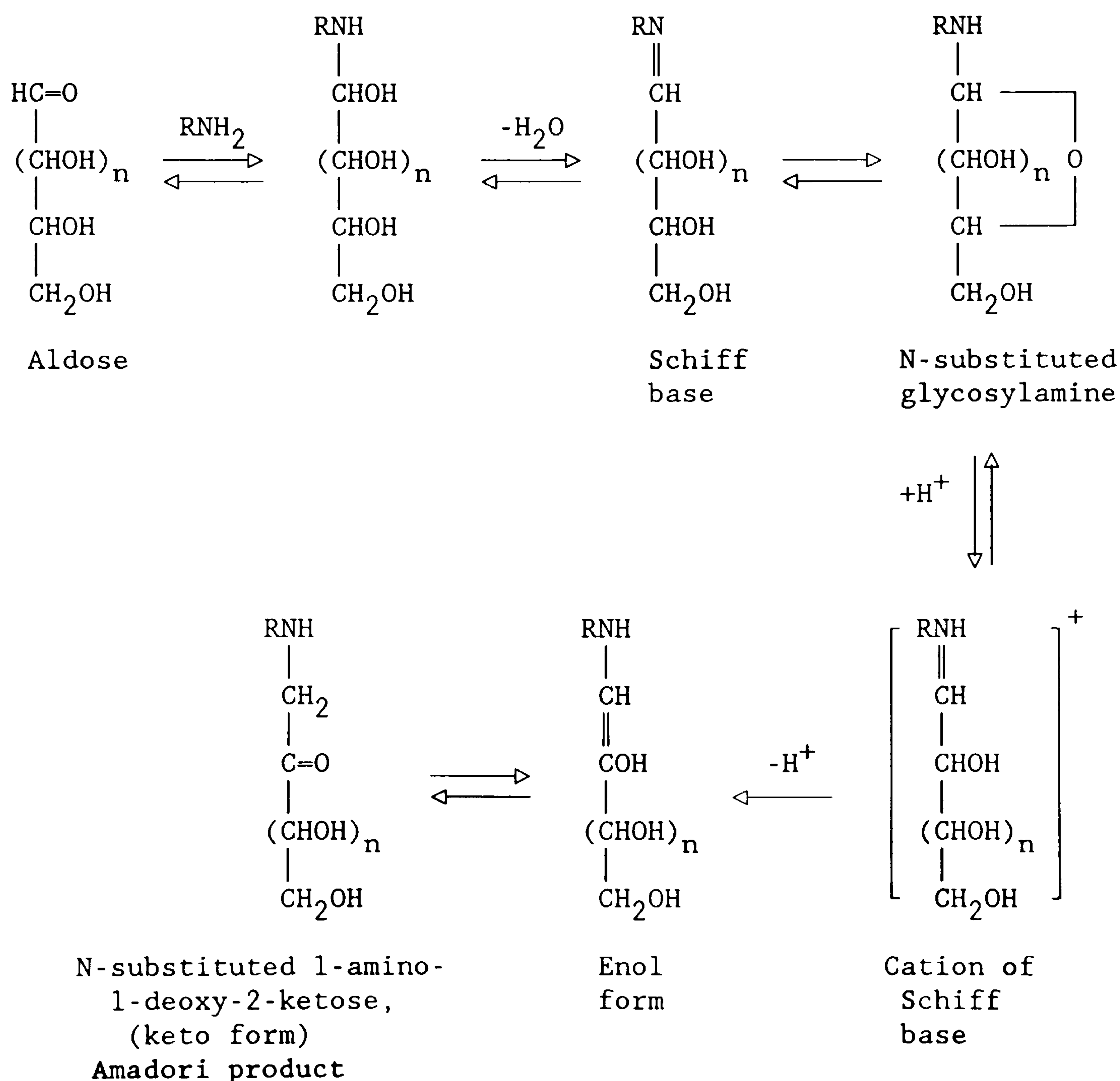


Figure 1.1B: Condensation of aldose sugars with amino compounds and Amadori rearrangement (Hodge 1953).

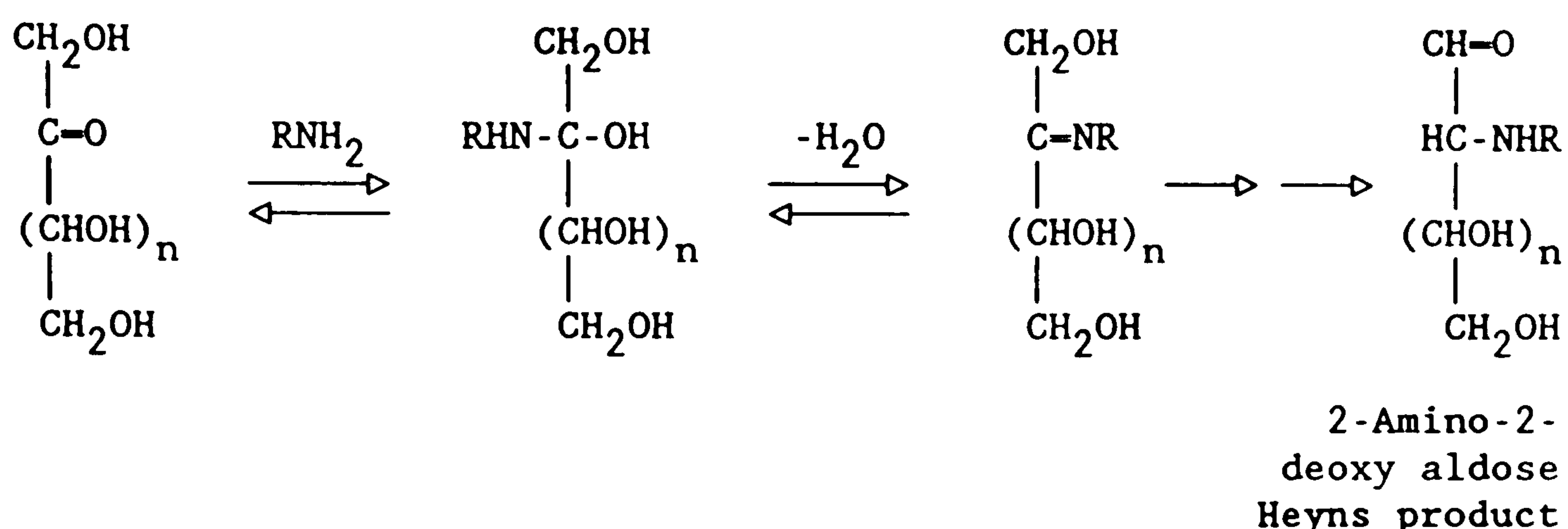


Figure 1.1C: Condensation of ketose sugars with amino compounds and Heyns rearrangement (Danehy 1986).

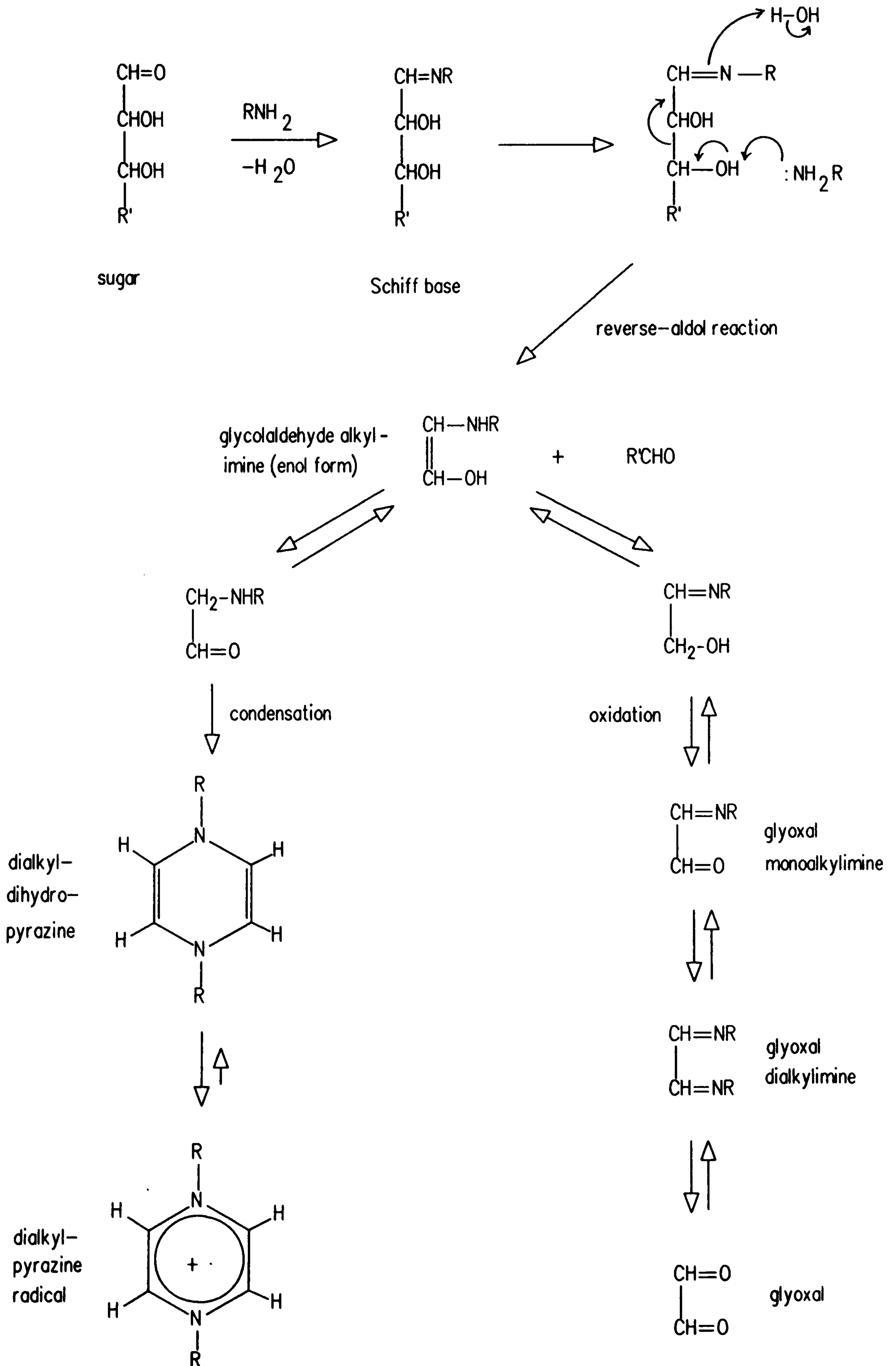
to its isomeric form, the 1-amino-1-deoxy-2-ketose (Amadori product); the amino acid itself may act as the catalyst (Vernin and Parkanyi 1982; Danehy 1986). Where a ketose, rather than an aldose sugar is the precursor, an analogous mechanism, known as the Heyns rearrangement, gives the 2-amino-2-deoxyaldose (Fig. 1.1C; Danehy 1986).

The intermediates and products of these reactions do not themselves contribute to flavour; however, their lability to heat ensures that they are important precursors for a wide range of flavour forming reactions.

Sugar fragmentation/free radical mechanisms

Namiki and Hayashi (1975) reported the development of free radicals in the early stages of the Maillard reaction. When an aqueous solution of a pentose sugar (D-arabinose) and β-alanine was heated at 100 ^{o}C, an ESR signal was observed immediately the reaction started. On the basis of the ESR signal these authors postulated that the free radical may arise from the elimination of a proton from the enaminol intermediate. More recently, free radical products have been identified as N,N'-disubstituted pyrazine cation radicals (Fig. 1.1D; Namiki and Hayashi 1983). Evidence suggests that these species are formed by the dimerization of 2-carbon enaminols from sugar fragmentation rather than from the enaminol products of the Amadori rearrangement.

Figure 1.1D: Possible pathways for the formation of dialkyl pyrazine radical and 2-carbon sugar fragments (Namiki and Hayashi 1983)



From these results it was concluded that an additional pathway of the Maillard reaction, involving sugar fragmentation at an early stage, can occur prior to or concurrent with the Amadori rearrangement (Namiki and Hayashi 1983). The suggested route of formation of the sugar fragments involves a retro-aldol type reaction of the Schiff base (formed following aldose-amino acid condensation; Fig. 1.1B) and is illustrated in Figure 1.1D.

The generation of sugar fragments is pH dependent, and is pronounced at alkaline pH, but negligible under acidic conditions (Hayashi and Namiki 1986). Furthermore, while free radicals were detected at pH 5-6 the greatest ESR signal intensity was observed at alkaline pH values; amino acid concentrations of 1.0 M were required to give detectable ESR signals (Namiki and Hayashi 1975). It was suggested that the Amadori rearrangement is the main pathway responsible for browning under acidic conditions but, at neutral or alkaline pH, the sugar fragmentation pathway described may account for the increased browning under these conditions (Hayashi and Namiki 1986). Thus, the importance of sugar fragmentation and free radical reactions in more dilute reaction mixtures at slightly acidic pH, such as those described later in this Thesis, is probably limited.

1.1.1.2 'Advanced' Maillard reactions

Later stages of the Maillard reaction involve the breakdown of the Amadori and Heyns products to give a range of different carbonyl compounds, which are capable of undergoing further reactions. The dicarbonyl compounds produced are important intermediates in the Strecker degradation of amino acids. Fragmentation reactions of some intermediates of these reactions and of sugars themselves also contribute compounds important for subsequent reactions.

Degradation of Amadori and Heyns compounds

Breakdown of the Amadori product via 1,2-enolization gives a 3-deoxyketose, which, in turn, yields a dehydroreductone; this cyclizes to give 5-hydroxymethyl-2-furfural or 5-methyl-2-furfural

Figure 1.1E: Further reactions of Amadori and Heyns products via 1,2-enolization (Hodge 1967; Mauron 1981; Vernin and Parkanyi 1982)

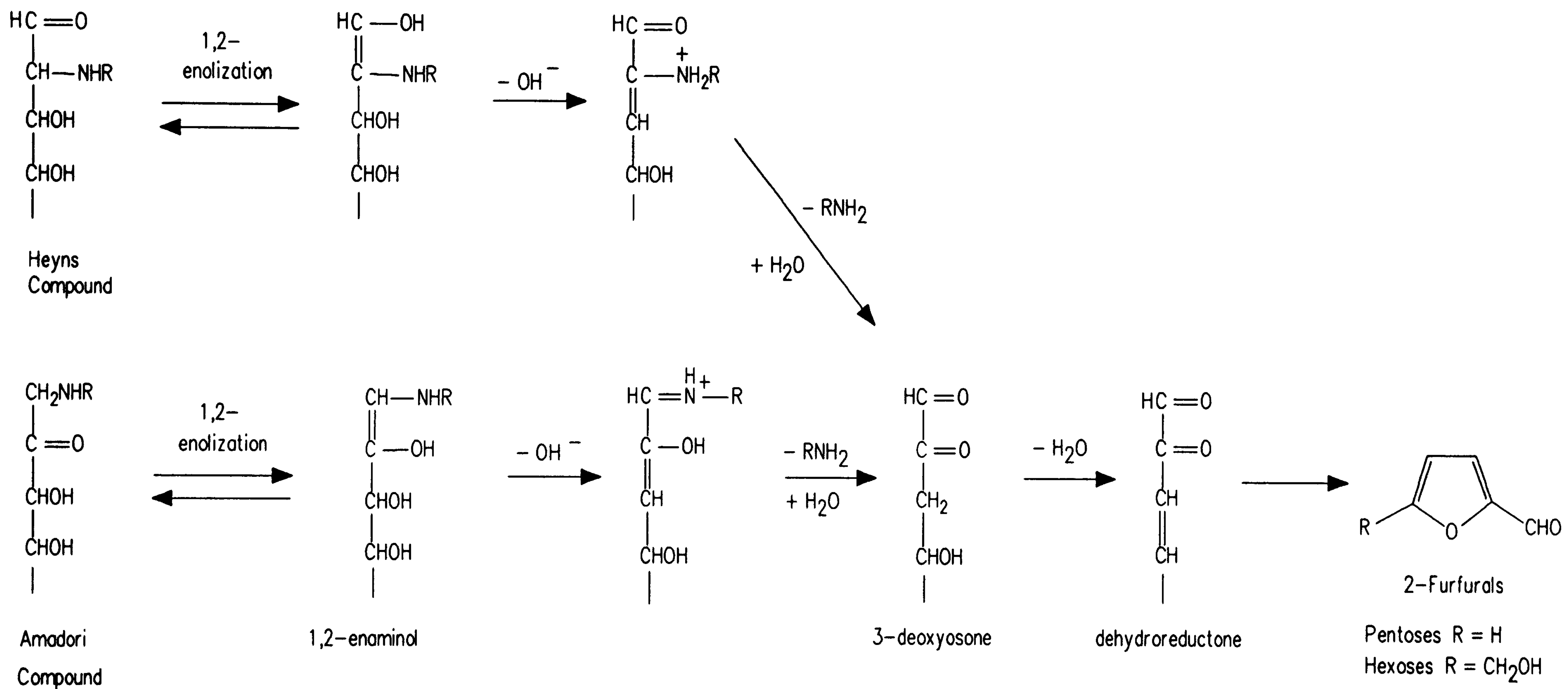
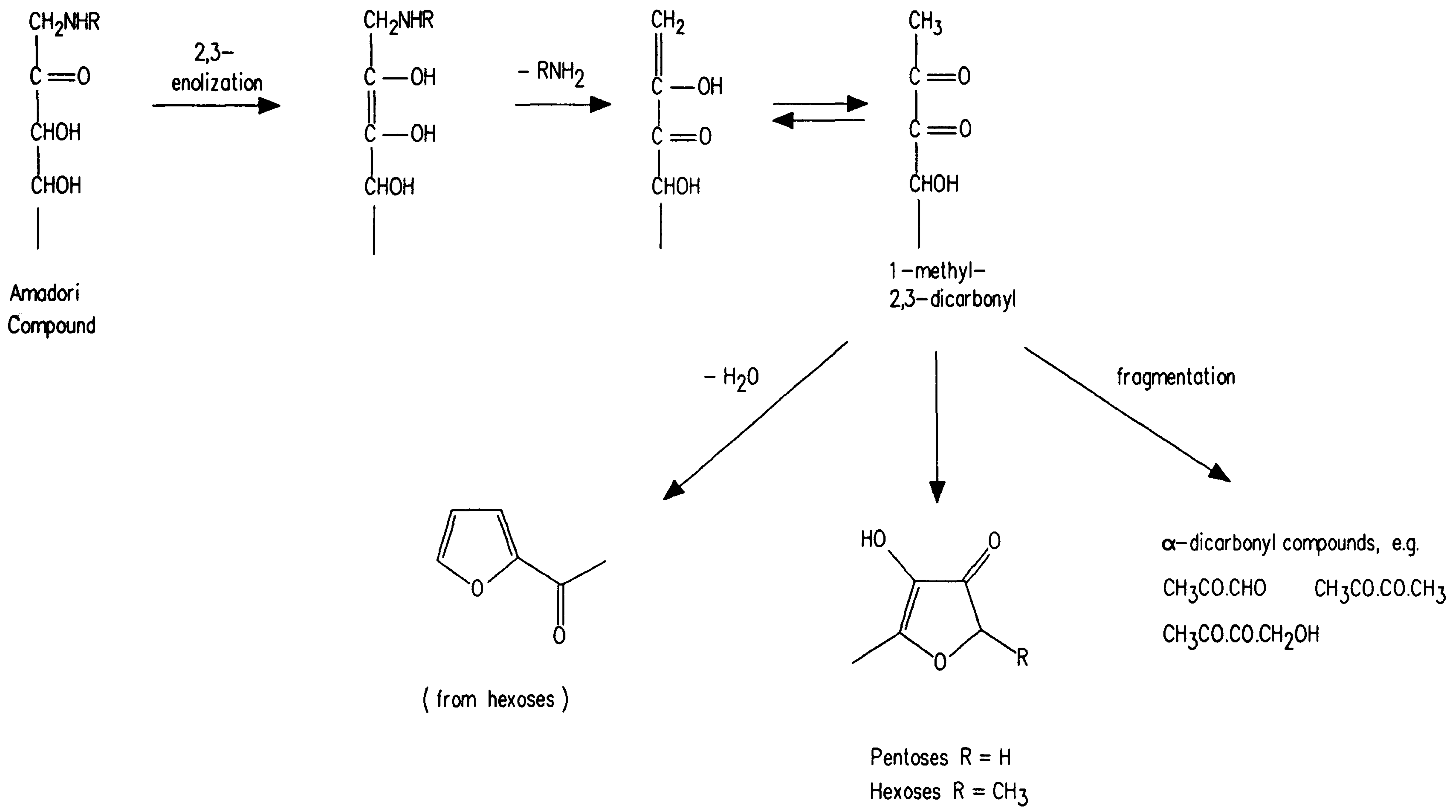


Figure 1.1F: Further reactions of Amadori product via 2,3-enolization (Hodge 1967)



from a hexose, or 2-furfural from a pentose sugar. The Heyns product can also give furfurals by a similar mechanism. These reactions are illustrated in Figure 1.1E.

An alternative pathway involves the 2,3-enolization of the Amadori product (Fig. 1.1F) and yields a 1-methyl-2,3-dicarbonyl compound and hence the 2,5-dimethyl- or 5-methyl-4-hydroxy-3(2H)-furanone, from hexose and pentose sugars, respectively (Hicks and Feather 1975; Tressl 1979). Related compounds, such as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (Mills *et al* 1970) and its isomer, 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2H)-furanone (Hiebl *et al* 1987) are also formed.

Degradation via 1,2-enolization is favoured in acidic systems, resulting in the increased formation of furfurals under these conditions (Hodge 1953), while more alkaline conditions tend to favour the formation of hydroxyfuranones via 2,3-enolization. It has been concluded that 1,2-enolization is promoted by a sufficiently acidic environment to allow protonation of the N atom, while partial unprotonation of the Amadori compound discourages this reaction and thus indirectly favours 2,3-enolization (Hicks and Feather 1975). The factors affecting these reactions have been discussed in detail by Feather (1981, 1989).

The degradation products formed from the Amadori and Heyns compounds may fragment by retro-aldolization pathways to give α-dicarbonyl compounds, such as pyruvaldehyde, diacetyl, glyoxal, hydroxyacetone and glyceraldehyde (Vernin and Parkanyi 1982). For example, the 3-deoxyosone, formed as a result of 1,2-enolization of the Amadori or Heyns product of glucose, gives pyruvaldehyde and 2,3-dihydroxypropanal by this reaction (Fig. 1.1G). The

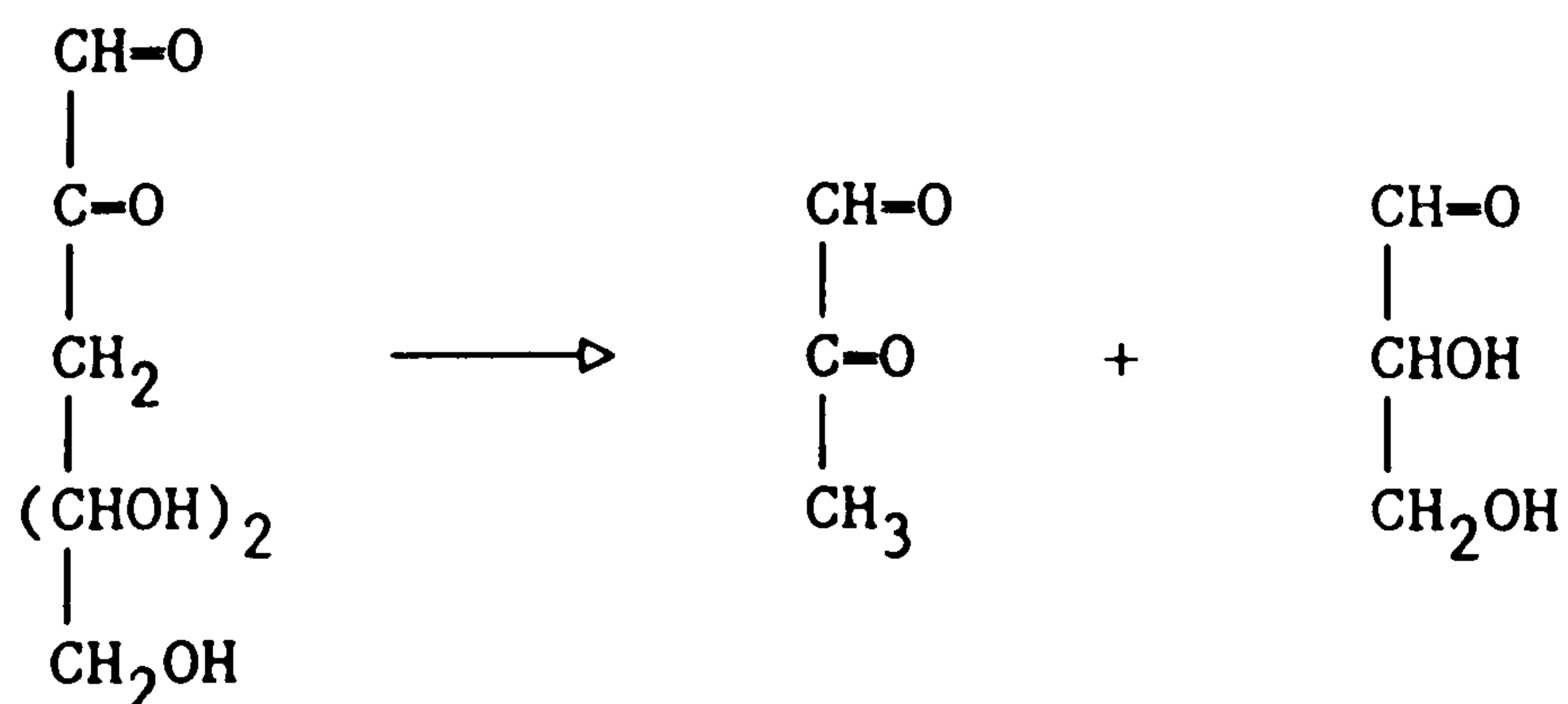


Figure 1.1G: Fragmentation of 3-deoxyosone of glucose by retro-aldolization reaction (Vernin and Parkanyi 1982).

products of such fragmentation reactions are reactive precursors for subsequent reactions, such as the Strecker degradation of amino acids.

Many of the products arising from the breakdown of Amadori or Heyns compounds are also formed during the caramelization of sugars (Hodge 1967). It is likely that the formation of volatile compounds, by reactions involving enolization or fragmentation, occurs in the absence of amines, but requires more extreme conditions of temperature or pH; the heating of sugars to about 300 ^{o}C yields a range of volatile products, including alkylfurans, furfurals, acetylfurans and furanones, pyrones, cyclopentenes as well as carbonyl, dicarbonyl and tricarbonyl compounds, with and without hydroxy substituents (Heyns *et al* 1966; Hodge 1967; Johnson *et al* 1969; Nursten 1980-1). Condensation with amines permits such reactions to take place under relatively mild conditions.

Strecker degradation

The Strecker degradation of amino acids involves the oxidative deamination and decarboxylation of amino acids by reaction with conjugated dicarbonyl compounds; these may be breakdown products of the Amadori or Heyns products or may be formed enzymatically (MacLeod and Seyyedain-Ardebili 1981).

Basic and hydroxy amino acids have been found to be the most reactive with α-dicarbonyl compounds, while the acidic amino acids react less strongly (Piloty and Baltes, 1979a). Proline and hydroxyproline (containing secondary amino groups) do not undergo the Strecker degradation reaction (Vernin and Parkanyi 1982).

Most amino acids undergo Strecker degradation by the mechanism illustrated in Figure 1.1H to give an aminoketone and an aldehyde with one fewer carbon atom than the parent amino acid. However, where the amino acid contains sulphur, additional products are formed; methionine yields NH_3, methional, methanethiol and dimethylsulphide (Schutte 1974; Vernin and Parkanyi 1982). The Strecker degradation of cysteine yields mercaptoacetaldehyde and an aminoketone by the same mechanism as that described above, while an alternative pathway gives NH_3, H_2S, acetaldehyde and

Figure 1.1H: Strecker degradation of α-amino acids

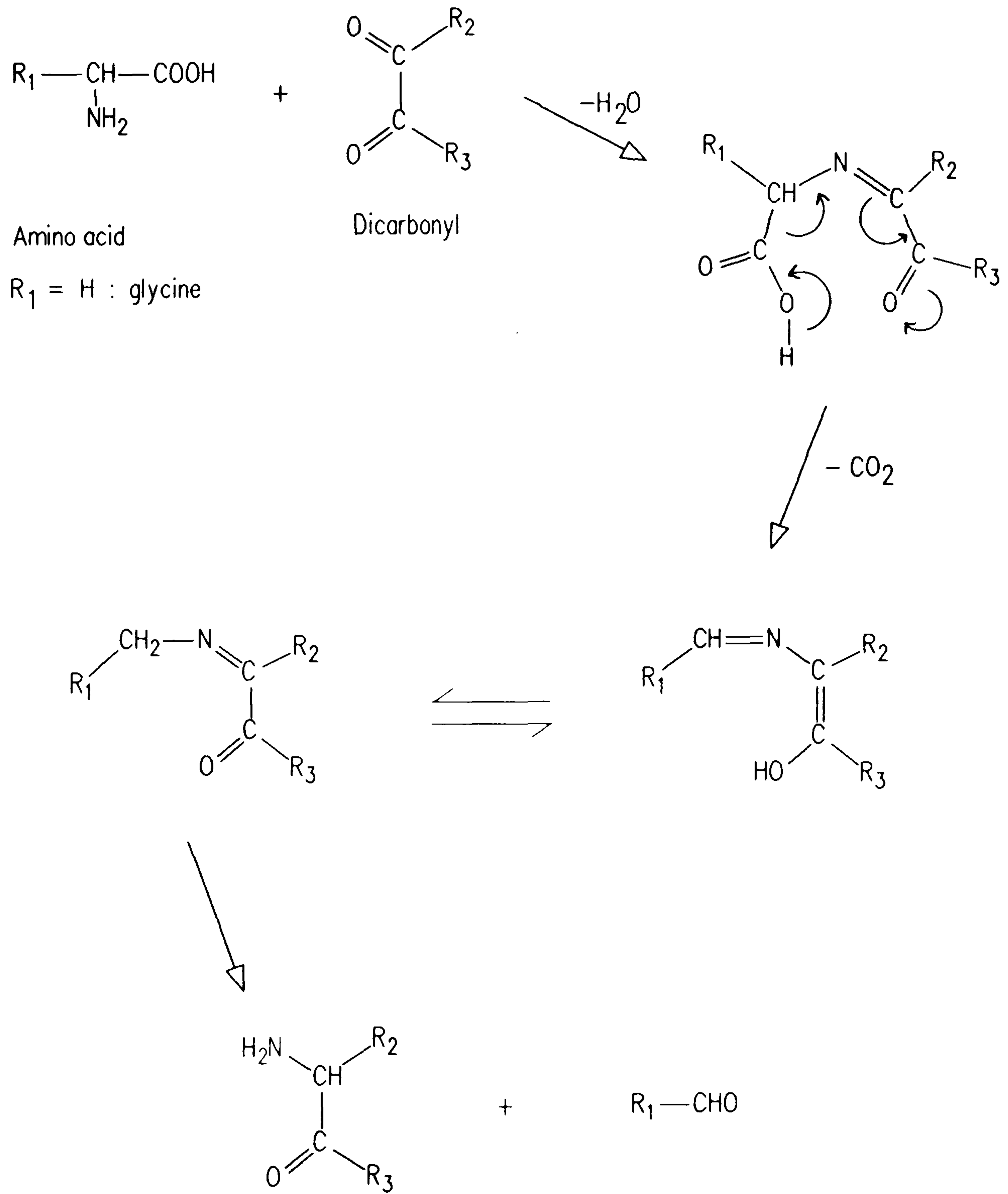
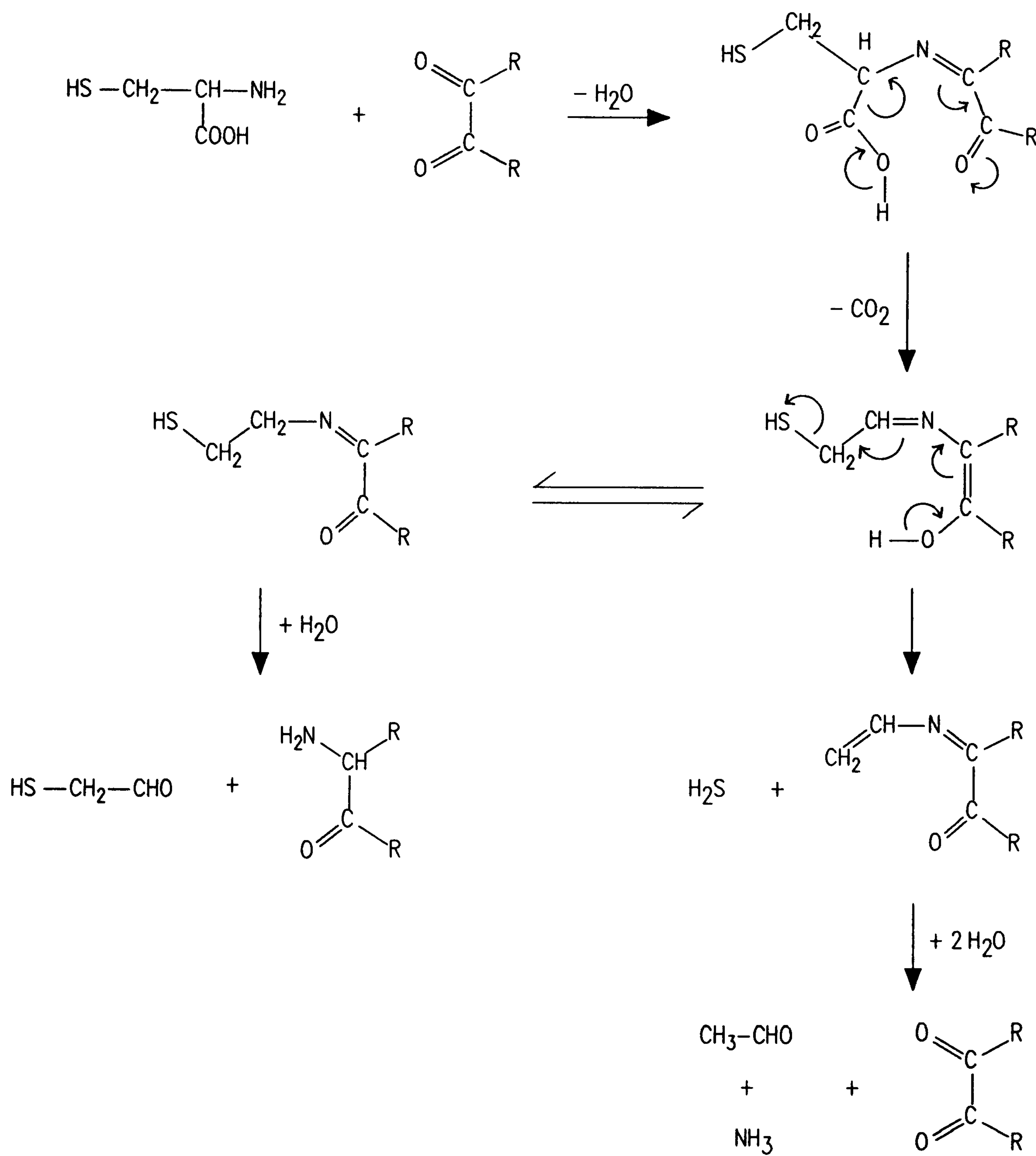


Figure 1.1I: Strecker degradation of cysteine



regenerates the original diketone (Fig. 1.1I; Kobayashi and Fujimaki 1965; MacLeod and Seyyedain-Ardebili 1981). These products are highly reactive and are involved in the formation of many sulphur and nitrogen-containing heterocyclic compounds.

Related degradation reactions between amino acids and unsaturated aldehydes and ketones have been reported by Rizzi (1976).

1.1.1.3 The formation of flavour volatiles.

While certain mechanisms have been established as being of primary importance in the 'early' and 'advanced' stages of the Maillard reaction, subsequent reactions are numerous and complex. Only those further reactions of importance for the formation of the main classes of heterocyclic compounds will be described here; mechanisms of formation of other compounds of special relevance to this work will be discussed at the appropriate juncture in later Chapters.

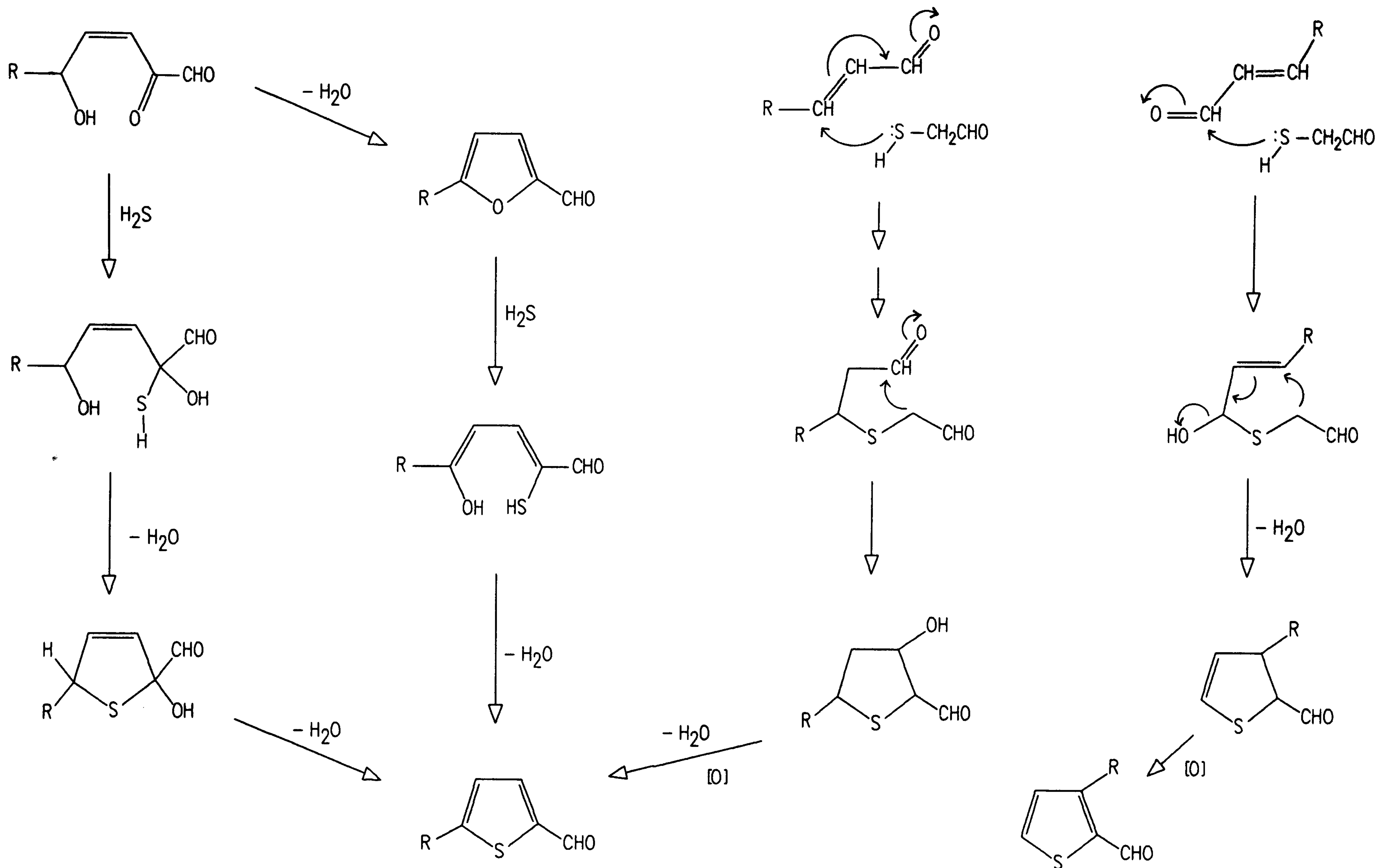
The mechanisms of formation of the major classes of heterocyclic products of the Maillard reaction have been reviewed by Mauron (1981), Vernin and Parkanyi (1982) and Hurrell (1982). The flavour significance of such compounds has been examined by Ohloff *et al* (1985) and Manley (1989), while the generation of volatile Maillard products from meat has been discussed by Mottram (1990).

Furans and thiophenes

Further reactions of the furfurals and hydroxy-3(2H)-furanones, arising from the degradation of Amadori or Heyns compounds, are responsible for the formation of many important aroma compounds.

2,5-Dimethyl-4-hydroxy-3(2H)-furanone is thermally unstable and its thermal degradation, alone, at 160 °C yields a range of products, including hydroxyketones and various 3(2H)-furanones with two or three alkyl (methyl or ethyl) substituents (Shu *et al* 1985c). It is proposed that the 2,5-dimethyl-3(2H)-furanone may arise by dehydration, and that opening of the ring structure, followed by retro-aldolization, gives 2 and 3 carbon

Figure 1.1J: 2-Formylthiophenes: some routes of formation (Mulders 1973; Shibamoto 1977; Vernin and Parkanyi 1982)



hydroxyketones; subsequent condensation reactions are thought responsible for the formation of hydroxypentanones and other alkyl-3(2H)-furanones.

Thiophenes may arise either by the reaction of sugar degradation products (dicarbonyl compounds, hydroxy-3(2H)-furanones or furfural) with H_2S, or by the thermal degradation of sulphur-containing amino acids alone (Vernin and Parkanyi 1982).

The formation of 2-formylthiophene in the reaction between 2-furfural, H_2S and NH_3 probably occurs by a ring opening mechanism involving exchange of the heteroatom (Shibamoto 1977). An alternative route of formation has been suggested for the 5- and 3-methyl-2-formylthiophenes, detected in a cysteine + ribose Maillard system, in which the thiol group of mercaptoacetaldehyde adds to the α,β-unsaturated systems of 2-butenal (Mulders 1973). The various mechanisms proposed for the formation of formylthiophenes are summarized in Figure 1.1J. Further reduction of such products may yield hydroxymethylthiophenes (Vernin and Parkanyi 1982). Alkyl substituted thiophenes can be formed by the cyclization of 1,4-diketones in the presence of H_2S (Fig. 1.1K; Boelens *et al* 1975).

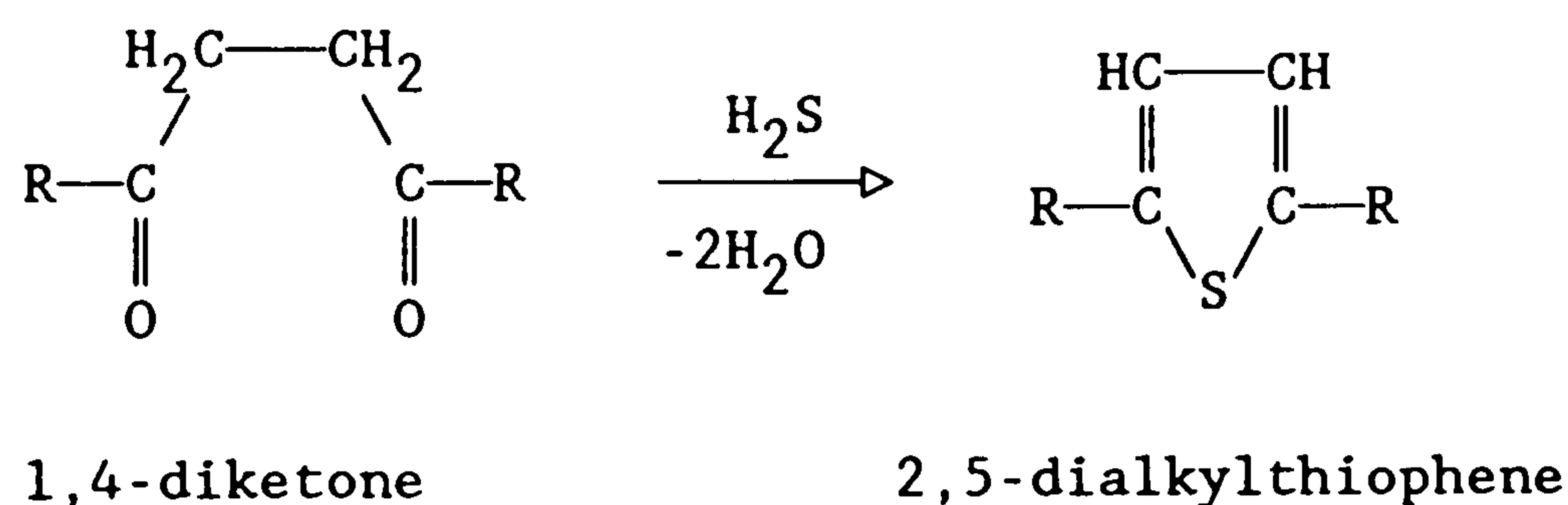
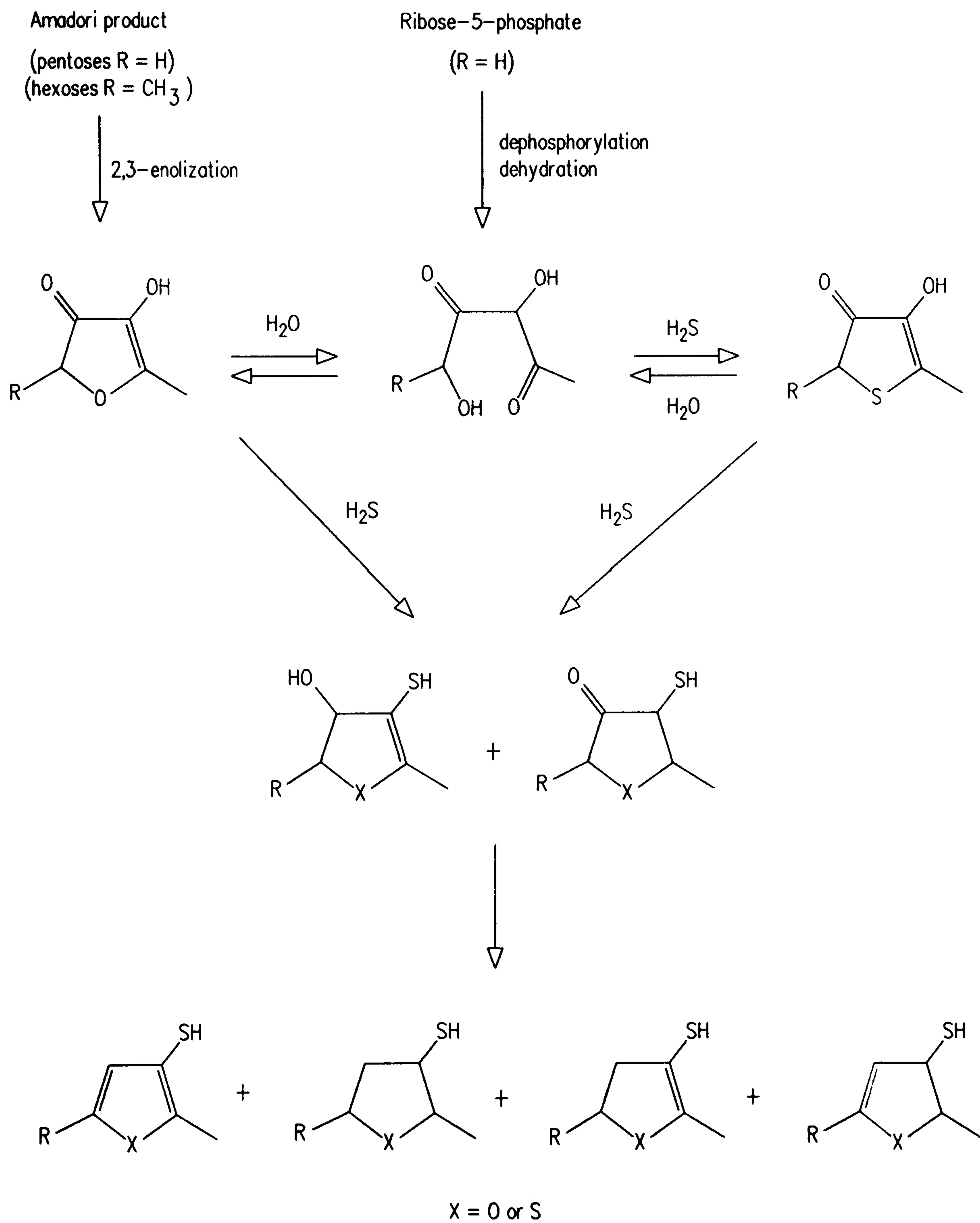


Figure 1.1K: Formation of alkylthiophenes from 1,4-diketones (Boelens *et al* 1975)

Both furfurals and hydroxyfuranones react with cysteine or H_2S to give furans and thiophenes with sulphur-containing substituents, some of which possess distinctive aromas and low odour thresholds. Products of the reaction between 2-furfural, H_2S and NH_3 include 2-furanmethanethiol and difurfuryl sulphide, formed by reaction of the aldehyde group with H_2S (Shibamoto 1977).

Figure 1.1L: Furanthiols and thiophenethiols: formation from 3(2H)-furanones and 3(2H)-thiophenones and hydrogen sulphide (van den Ouweland and Peer 1975)



The reaction of 5-methyl-4-hydroxy-3(2H)-furanone with H_2S gives saturated and unsaturated furanthiols and thiophenethiols as well as mercapto- and hydroxy-3(2H)-thiophenones (van den Ouweland and Peer 1975). Many of these compounds possess distinctive odours, with several reminiscent of roast meat. The suggested pathways for the formation of such compounds are illustrated in Figure 1.1L. As 4-hydroxy-5-methyl-3(2H)-furanone can be formed from ribose-5-phosphate via dephosphorylation and dehydration, it is likely that these compounds may also be formed by routes not involving the Maillard reaction (van den Ouweland and Peer 1975).

Pyrroles

The reaction between 2-furfural, H_2S and NH_3 not only yields the sulphur-containing compounds already described, but also nitrogen-containing compounds such as 2-formylpyrrole, 2-furylcyanide and N-furfuryl-2-aminofuran, indicating that both the aldehyde group and the heteroatom may react with NH_3 in a similar way to that described for H_2S (Shibamoto 1977).

A possible mechanism for the formation of 2-acetylpyrrole and 2-formyl-5-methylpyrrole, with or without N-alkyl substituents, involves the cyclization of condensation products formed from amino acids and the 1,2-dicarbonyl derivatives of aldose and ketose sugars (Kato and Fujimaki 1968; Shaw and Berry 1977). Nyhammar *et al* (1983) propose routes for the formation of pyrroles (and pyridines) from intermediates in the reaction between glucose and glycine (Fig. 1.1M). An alternative mechanism has been suggested by Rizzi (1974) in which the 5-carbon of a 2-acylfuran is subjected to nucleophilic attack by the amino group of an amino acid to give N-alkyl-2-acylpyrroles (Fig. 1.1N).

A series of unusual pyrroles, including bicyclic compounds, have been reported in reaction mixtures containing proline or hydroxyproline and mechanisms suggested for their formation (Tressl *et al* 1985a,b, 1986). The formation of pyrroles in these and other model systems has been reviewed by Maga (1981).

Figure 1.1M: Pyrroles and pyridines: proposed routes of formation from glucose and glycine (Nyhammar *et al* 1983)

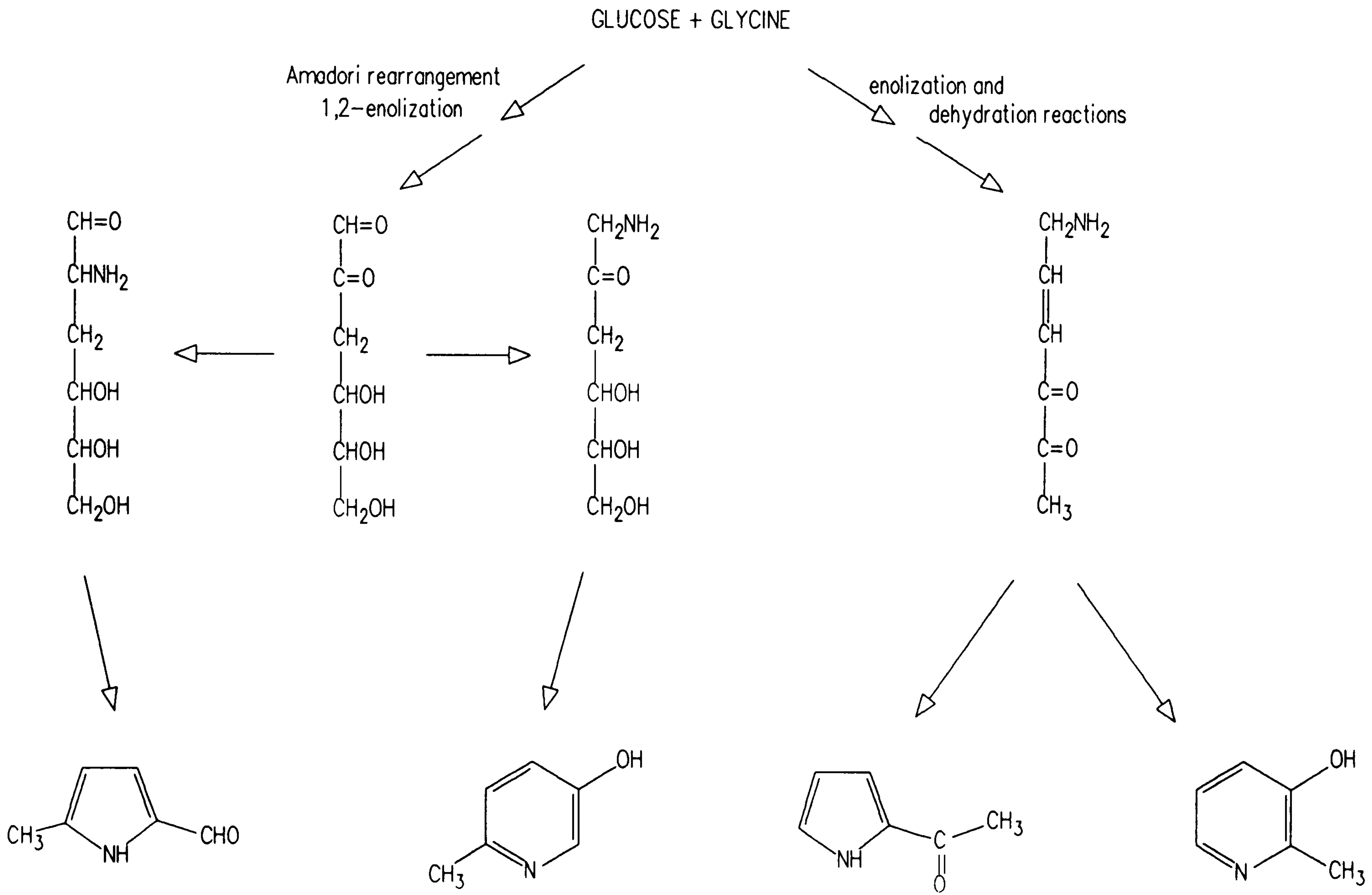
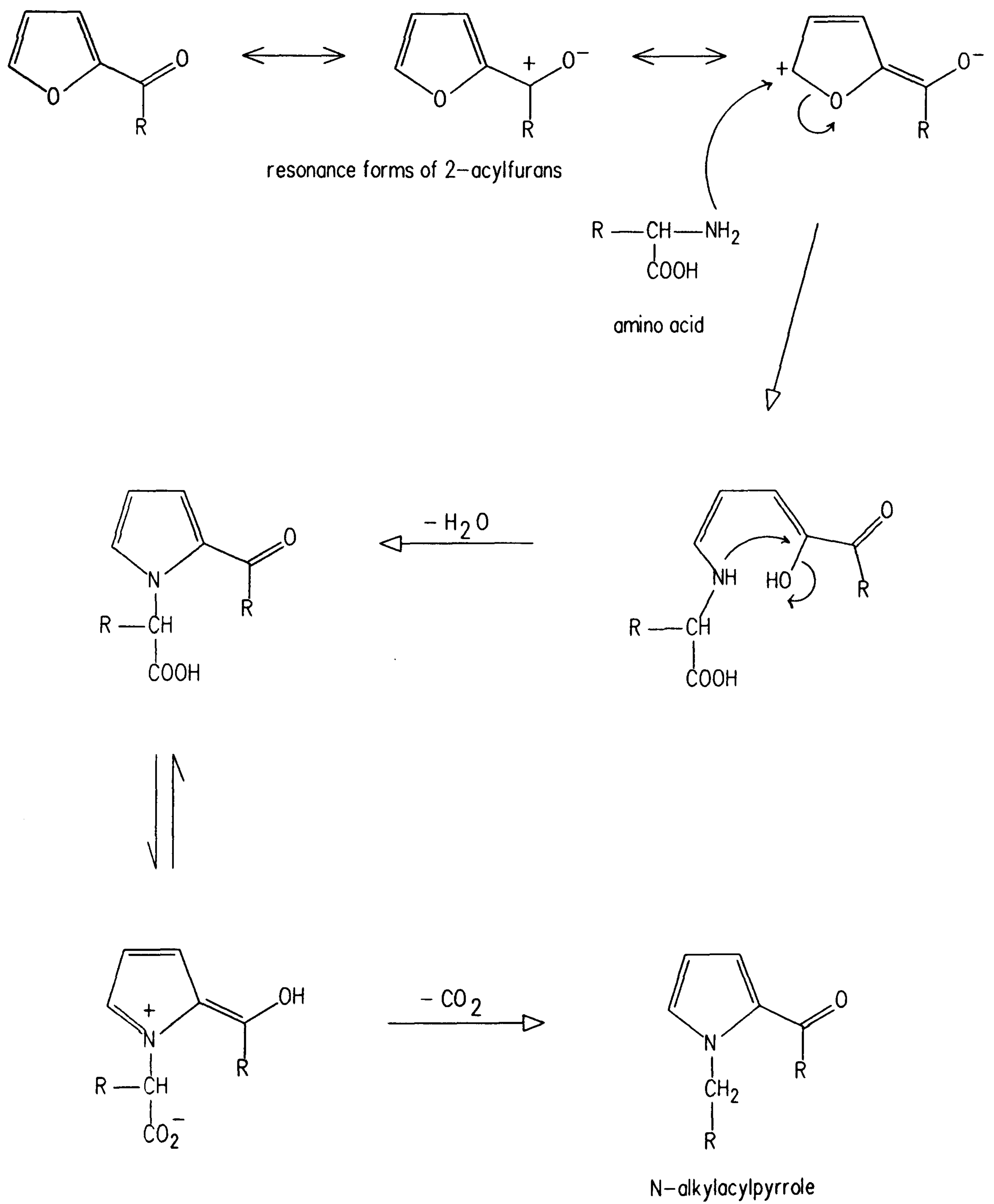


Figure 1.1N: Mechanism for the formation of N-alkylacylpyrroles (Rizzi 1974)



Pyridines

The mechanisms of formation of pyridines from intermediates of the Maillard reaction have been less thoroughly investigated than those of other Maillard products. The suggested pathways generally involve the condensation of aldehydes with NH_3 (Vernin and Parkanyi 1982; Hurrell 1982). Lien and Nawar (1974a,b) detected alkylpyridines amongst the thermal degradation products of alanine, but not valine, leucine or isoleucine and suggested mechanisms of formation involving several condensation reactions between NH_3 and acetaldehyde. Suyama and Adachi (1980, 1986) have proposed a mechanism for the formation of pyridines which involves the condensation of three molecules of an aldehyde with an amino acid or a primary amine to give pyridinium betaines, which decompose on heating to give alkyl substituted pyridines (Fig. 1.10).

The generation of NH_3 during the Strecker degradation of cysteine promotes the formation of pyridines in model systems containing this amino acid (Vernin and Parkanyi 1982). However, a Maillard reaction containing lysine, which possesses an ϵ-amino group, produces higher quantities and a greater variety of pyridines (Whitfield *et al* 1988).

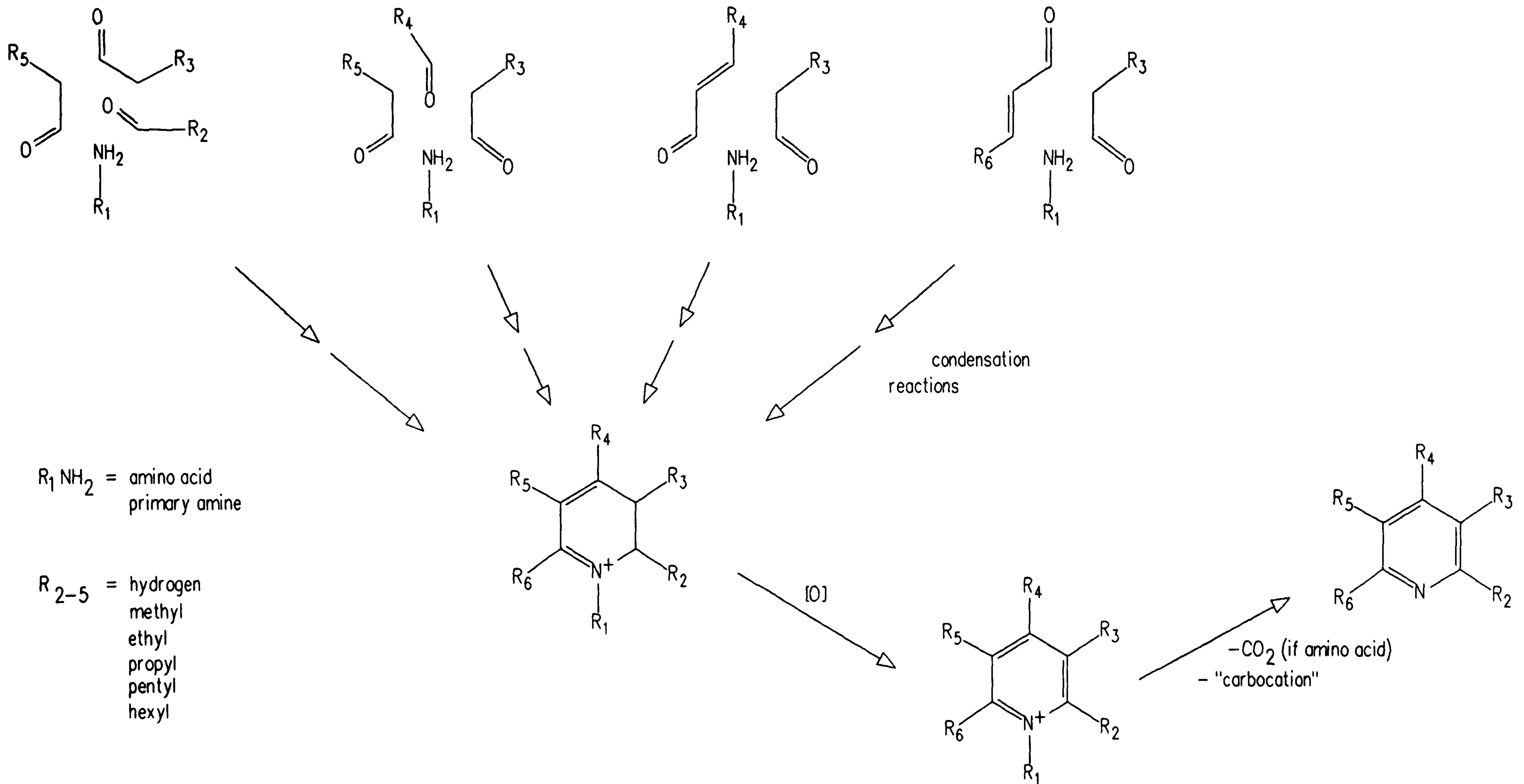
Pyridines can also be formed by the thermal treatment of other heterocyclic compounds, such as N-substituted pyrroles, from furfural or 2-acetylfurans heated with NH_3 or from the reaction of oxazoles and thiazoles with dienes (Vernin and Parkanyi 1982).

Pathways for the formation of hydroxy pyridines by cyclization of condensation products from glycine and glucose have been proposed (Nyhammar *et al* 1983), as illustrated in Fig. 1.1M.

Pyrazines

Pyrazines are important products of the Maillard reaction and their mechanisms of formation have been the subject of many studies, both using amino acid-sugar model systems and simpler reactions between carbonyl compounds and NH_3; these have been reviewed by Maga (1982) and Vernin and Parkanyi (1982).

Figure 1.10: Formation of alkyl substituted pyridines from aldehydes and primary amines (Suyama and Adachi 1980, 1986)



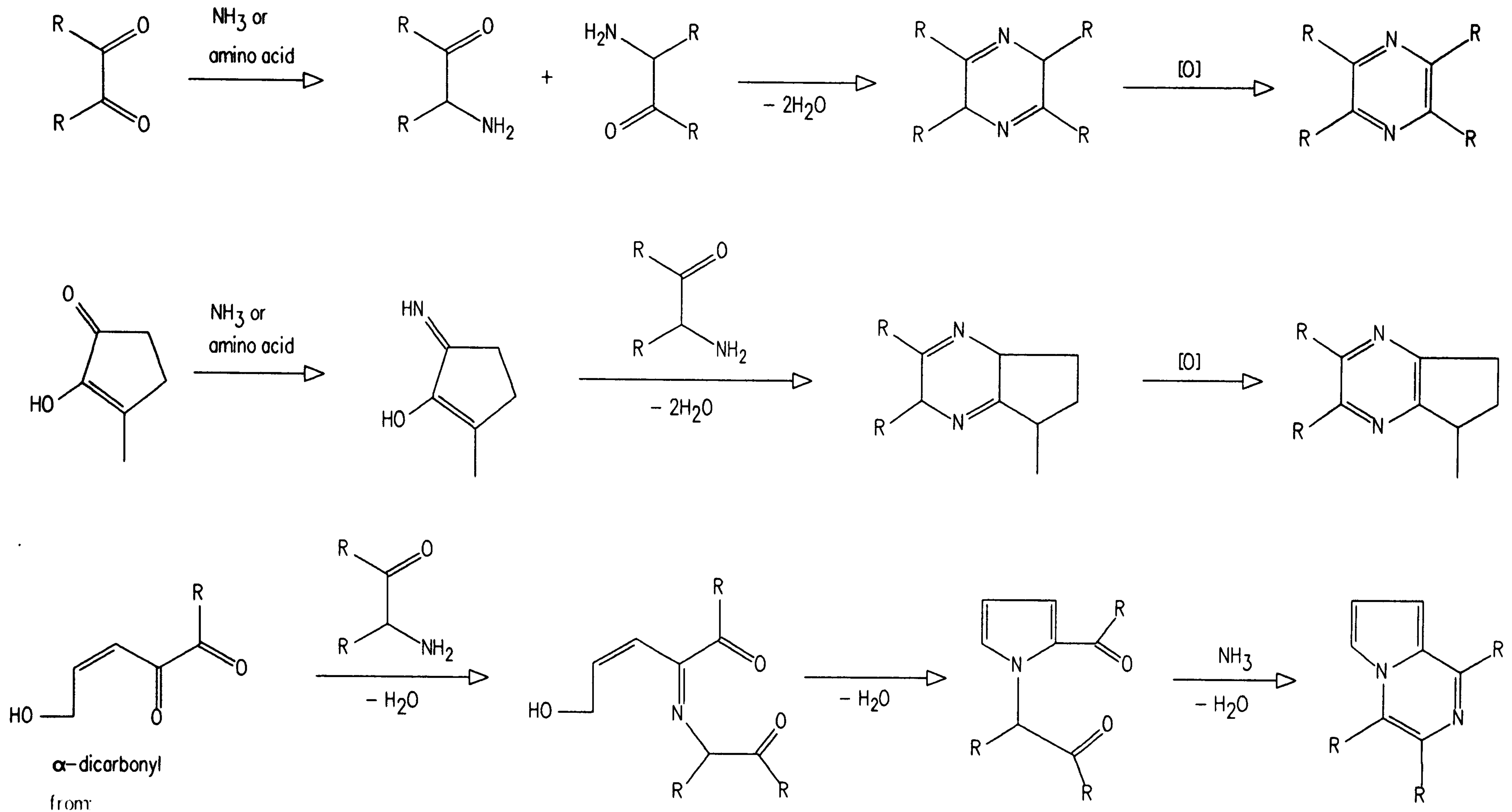
An important route of formation of alkyl-substituted pyrazines is by the condensation of two aminoketones, such as those arising from the Strecker degradation of amino acids, to give a dihydropyrazine which may then be oxidized to the pyrazine (Fig. 1.1P; Dawes and Edwards 1966). Shibamoto and Bernhard (1977) studied model systems containing different sugars and ammonia and explained the formation of most of the pyrazines detected in terms of reactions between sugar fragments and NH_3.

Aminocarbonyl compounds may also react with cyclopentenone derivatives and NH_3 to give dehydrocyclopentapyrazines, or with dicarbonyl derivatives of sugars to give pyrrolopyrazines (Fig. 1.1P; Flament *et al* 1976, 1977; Shibamoto and Bernhard 1978).

The nature of substituents of the amino ketones are dependent on the structure of the parent dicarbonyl compound, not the amino acid, which supplies only the nitrogen (Koehler *et al* 1969). Indeed, it has been proposed that free NH_3, rather than the amino acid itself, may be the key intermediate in pyrazine formation (Newell *et al* 1967; van Praag *et al* 1968); both groups reported that, qualitatively, the same pyrazines were formed regardless of the amino acid employed. However, Koehler *et al* (1969) reported that the N-source does affect the pyrazines produced, suggesting that the NH_2 group is still attached to the amino acid during the condensation reaction. More recent studies, aiming to clarify these discrepancies, have compared the products of reactions between glucose and glycine, monosodium glutamate or various ammonium salts (Wong and Bernhard 1988). The nature of the N-source was found to have a pronounced effect both on the nature and amounts of pyrazines formed; while some differences could be attributed to the generation of additional carbonyl compounds from the Strecker degradation of the amino acids, reasons for the differences between the systems containing different ammonium salts were not clear. Huang *et al* (1989) also found the distribution of pyrazines to be affected by the nature of the amino acid used in an amino acid-glucose systems.

Yuan *et al* (1989a,b) have suggested mechanisms of pyrazine formation which do not involve Strecker degradation to give aminoketones; it is proposed that condensation products of amino acids with 1,2-dicarbonyl derivatives of sugars undergo further

Figure 1.1P: Formation of pyrazines from Maillard intermediates (Flament *et al* 1976, 1977; Maga 1982)



condensation reactions to give a number of substituted dihydropyrazines and pyrazines.

Although small amounts of pyrazines are detected in model systems at temperatures less than 70 ^oC, the generation of pyrazines generally requires more elevated temperatures (Shibamoto and Bernhard 1976). Above 70 ^oC the rate of pyrazine formation increases rapidly to a maximum at 120 ^oC. However, at higher temperatures, and reaction times greater than 4 h, pyrazine formation declines. Pyrazine production has been found to increase with pH over the range pH 5.0 to 9.0 and over the range of water activities, a_w = 0.32 to 0.75 (Leahy and Reineccius 1989). However, further increases in water content have been found to decrease pyrazine formation, presumably due to dilution of the reactants.

Oxazoles and thiazoles

Both oxazoles and thiazoles can be formed in reaction mixtures containing 1,2-dicarbonyl compounds, acetaldehyde, H_2S and NH_3 (Boelens *et al* 1975). Oxazolines and oxazoles may be formed by the condensation of either α-aminoketones (from Strecker degradation) with aldehydes, or of hydroxy ketones with imines (Figure 1.1Q; Mussinan *et al* 1976; Vernin and Parkanyi 1982). For instance, 2,4,5-trimethyl-3-oxazoline can be formed in the reaction between acetaldehyde, 3-hydroxy-2-butanone and ammonia (Mussinan *et al* 1976). In a similar reaction, mercaptoketones, from the reaction of dicarbonyl compounds with H_2S, can undergo condensation reactions with imines to give thiazolines and thiazoles (Figure 1.1R; Takken *et al* 1976).

The formation of 2,4-dimethyloxazole, as well as furans and pyrroles containing more than five carbon atoms from the reaction between 2-furfural, H_2S and NH_3 indicates that 2-furfural fragments into smaller units, capable of recombining by condensation reactions (Shibamoto 1977).

The reaction of a hydroxylated amino acid (eg. serine, threonine) with an aldehyde can give dialkyloxazoles by the reaction shown in Figure 1.1S. If a 1,2-dicarbonyl compound reacts in place of the aldehyde, a similar pathway yields the 2-acyloxazole (Vitzthum and

Figure 1.1Q: Oxazoles and oxazolines: some mechanisms of formation (Mussinan et al 1976; Vernin and Parkanyi 1982)

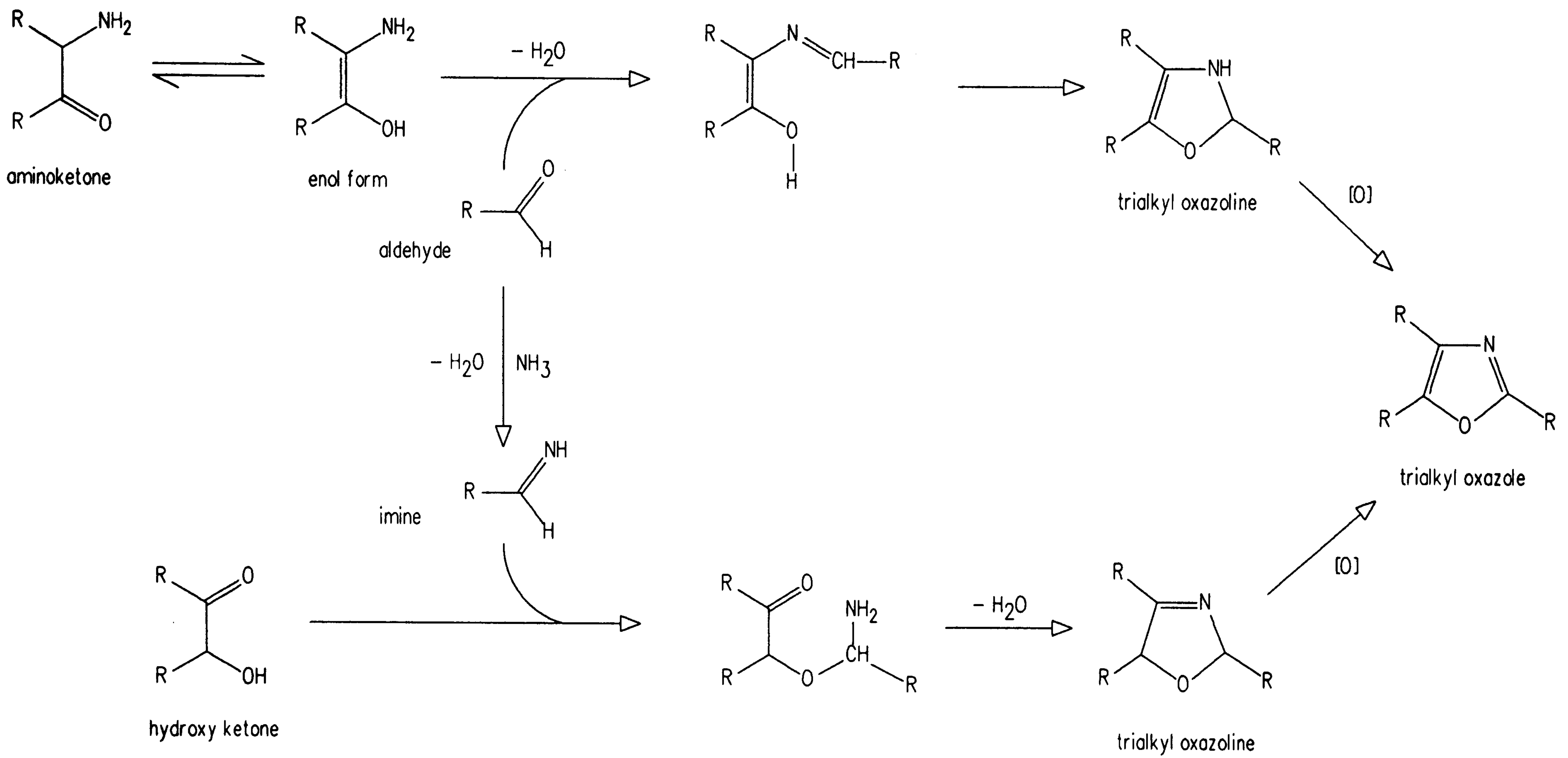


Figure 1.1R: Thiazoles and thiazolines: mechanisms of formation (Takken *et al* 1976)

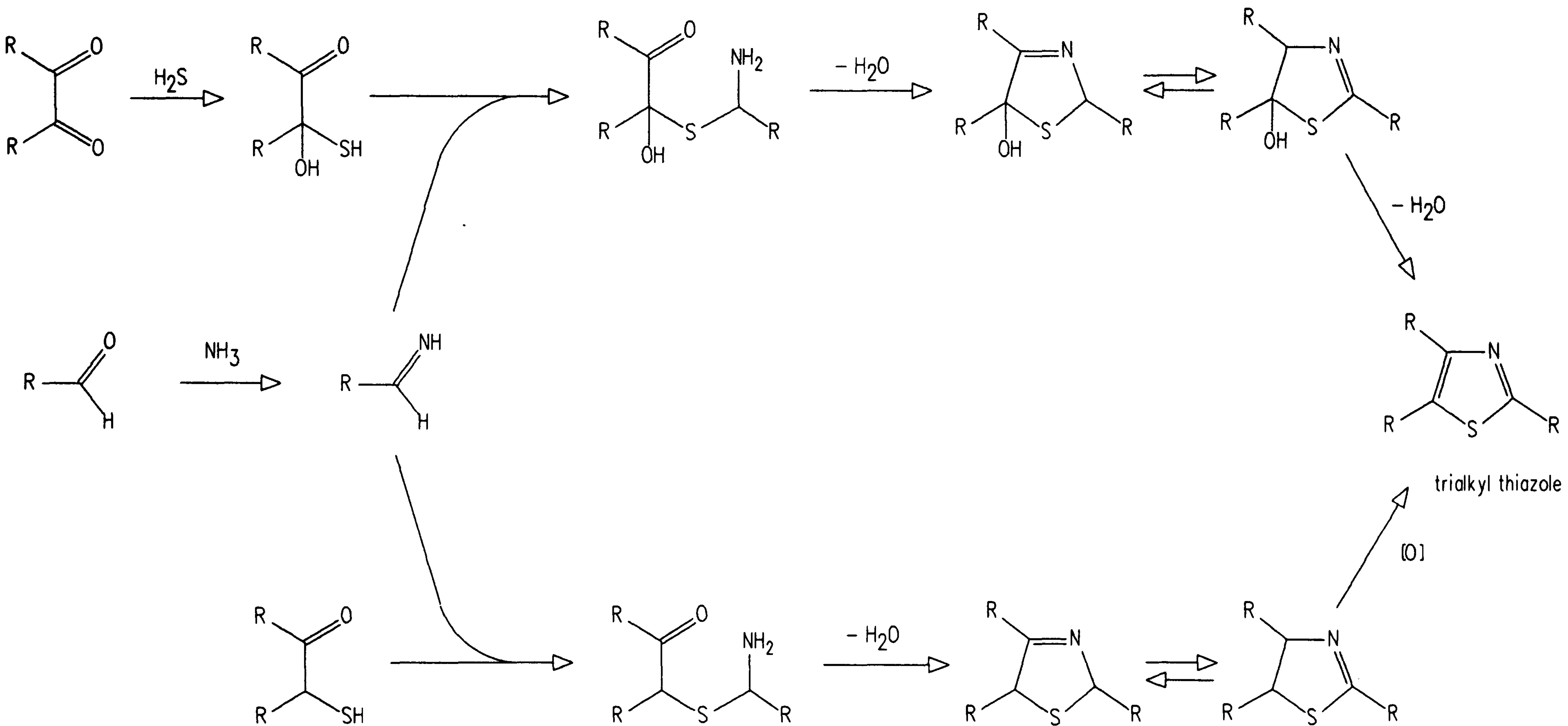


Figure 1.1S: Mechanism of formation of dialkyl oxazoles from amino acids and aldehydes (Vitzthum and Werkhoff 1974b)

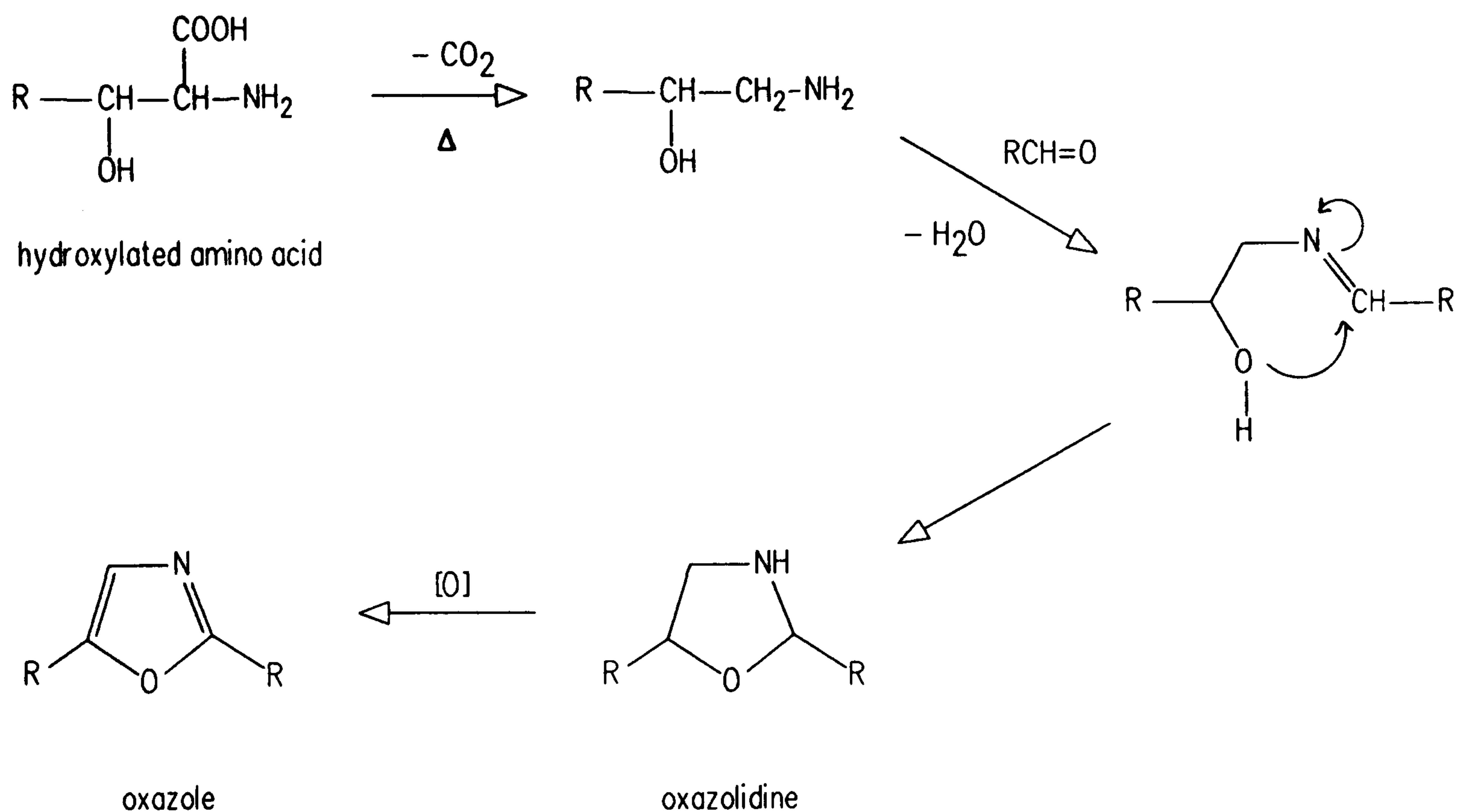
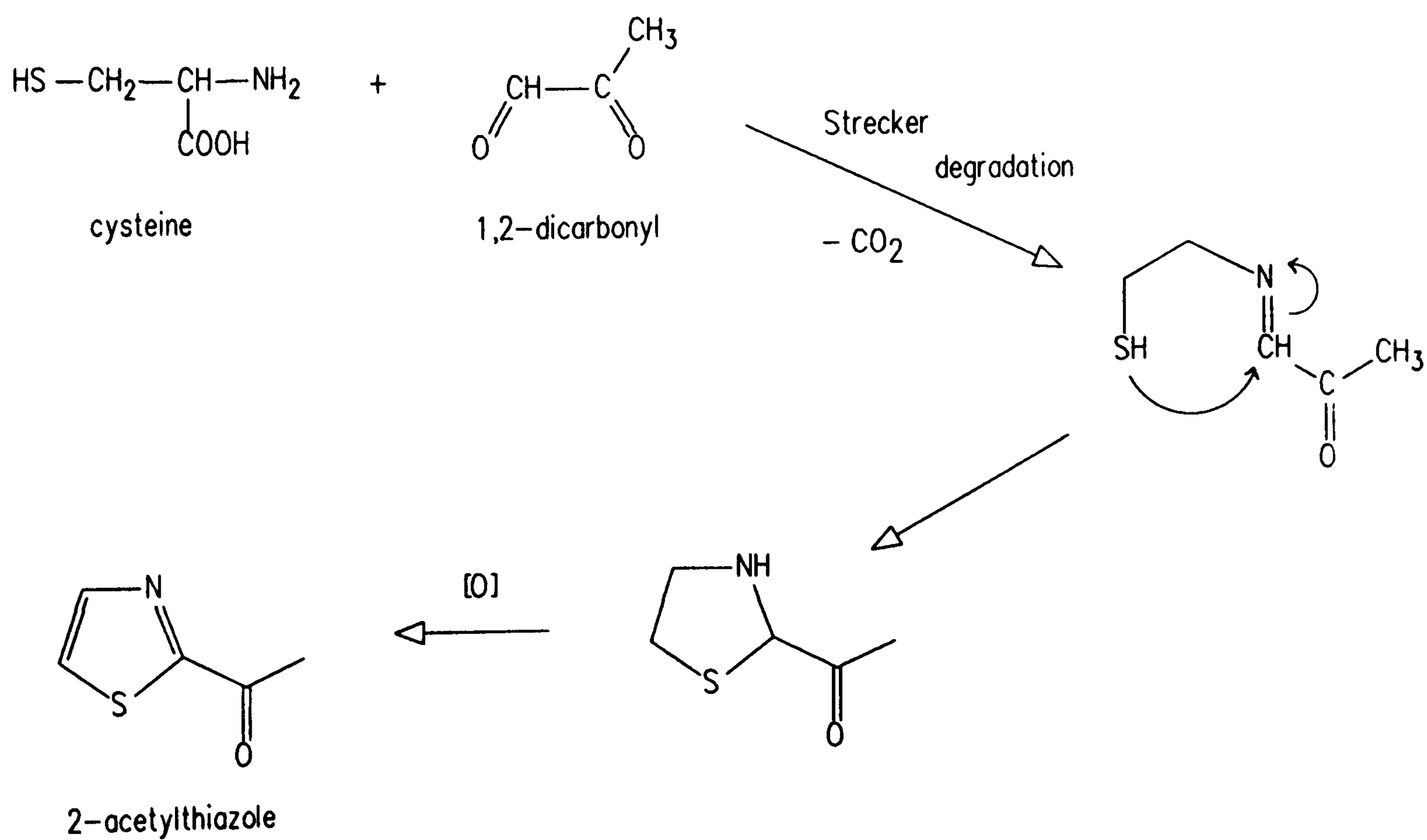


Figure 1.1T: Pathway for the formation of 2-acetylthiazole (Mulders 1973)



Werkhoff 1974b). A mechanism for the formation of 2-acylthiazoles from cysteine and a 1,2-dicarbonyl has been suggested by Mulders (1973; Fig. 1.1T); the initial stages are the same as those of the Strecker degradation of cysteine, but the condensation and decarboxylation steps are followed by cyclization rather than fragmentation.

Thiazoles, thiazolines and thiazolidines are formed during the thermal degradation of cysteine alone (Fujimaki *et al* 1969; Kato *et al* 1973a; Shu *et al* 1985a); the 2-alkyl substituted compounds may arise by condensation reactions between the decarboxylated form of cysteine and, for example, acetaldehyde (Fujimaki *et al* 1969; Sheldon and Shibamoto 1987).

1.1.2 FACTORS AFFECTING THE RATE OR DIRECTION OF THE MAILLARD REACTION

1.1.2.1 Temperature

The early stages of the Maillard reaction proceed spontaneously at low temperatures (Danehy 1986). However, elevated temperatures are required for 1,2- and 2,3-enolization and Strecker degradation reactions; most studies on these reactions have been performed at temperatures of 80 °C and above (Hodge 1953; Hodge 1967; Vernin and Parkanyi 1982) although these reactions do occur more slowly at lower temperatures.

It was observed by Maillard (1916) that the rate of the Maillard reaction was temperature-dependent and this observation has since been confirmed by other workers (Hurrell 1982). The rate of reaction, measured in terms of decrease in amino-N content, was 40,000 times greater at 70 °C than at 0 °C and increased uniformly from 0 to 90 °C (Lea and Hannan 1949). The time of heating also affects the progress of reaction; a similar loss of lysine ϵ-amino groups can be observed in an albumin-glucose system heated at 37 °C for 30 days as is observed following heating at 121 °C for 15 mins. However, at the lower temperature this loss is thought to be due to the formation of Amadori compounds; aroma volatiles and browning products generally require higher temperatures for their formation (Hurrell and Carpenter 1974).

More recent studies have evaluated quantities of selected volatiles produced in a proline-rhamnose model system at four temperatures ranging from 139 to 190 ^{o}C; while some compounds increase over the whole range, others reach a maximum at about 150 ^{o}C (Shaw and Ho 1989).

1.1.2.2 Water content

The Maillard reaction proceeds most readily at low moisture contents and more slowly in aqueous solution (Hurrell 1982). Various workers have investigated the effect of moisture on the development of browning and it appears that melanoidin formation is maximized at a water content of approximately 30% (Hurrell 1982). Very low water contents or complete dehydration retard or stop the reaction. Optimal conversion to rearrangement products (N-glycosylamines) takes place at a water content of 25-35% (Vernin and Parkanyi 1982).

Hartman *et al* (1984b) have shown that water content can also affect the amount and type of volatile products formed. The maximum yield of volatiles from an ascorbate, glutamate, cystine, thiamine model system was obtained at a water activity of 0.72 (3:1, water : propylene glycol); sulphur containing compounds predominated at $a_w > 0.4$ while products of dehydration type reactions were favoured by lower moisture levels.

1.1.2.3 pH and buffers

The overall rate of reaction, as determined by rate of loss of amino-N or rate of browning, is increased by a rise in pH (Lea and Hannan 1949; Pomeranz *et al* 1962; El Ode *et al* 1966; Shinohara *et al* 1986). It has been suggested that the increased browning observed at alkaline pH values may be a result of the increased importance of sugar fragmentation reactions in the early stages of the Maillard reaction (Hayashi and Namiki 1986). However, the effect of pH depends on the method used to monitor progress of reaction; when total carbonyls produced at 100 ^{o}C was measured, the amount was found to be similar at pH 5.0, 6.5 and 8.0 (El'Ode *et al* 1966). The total yield of volatiles from a model reaction

between 2,5-dimethyl-4-hydroxy-3(2H)-furanone and cystine has been found to be higher at pH 4.7 than pH 2.4 or pH 7.0 (Shu and Ho 1989); pH 4.7, the isoelectric point of cystine, also yields the most trithiolanes and thiophenes. Similar results have been obtained from cystine heated alone (Shu *et al* 1985b; Sec. 1.1.3.2).

Hayase and Kato (1986) have also reported that different classes of compounds respond differently to changes in pH and concluded that the compounds formed at pH 11.4 comprised mainly fission products of glucose while, at pH 4.0, heterocyclic compounds showing no C-C fission were formed; at pH 6.5 both types of product were observed. These results are corroborated by those of Shinohara *et al* (1986) who found that the highest levels of 5-hydroxymethylfurfural and furfural were formed under acidic conditions. The propensity to form small dicarbonyl compounds at elevated pH values, together with the increased amounts of unprotonated amino groups, probably explains why the formation of pyrazines is favoured under these conditions.

Most of these studies have examined the effects of relatively large differences in pH. Recent studies show that the formation of volatile products (Mottram and Leseigneur 1990; Farmer and Mottram 1990b) and colour (Mottram *et al*, unpublished results) are sensitive to pH differences of as little as 0.5 pH unit.

The use of phosphate buffer is known to accelerate browning (Spark 1969). Saunders and Jervis (1966) have suggested that buffering tends to hold the pH at a value favouring rapid reaction; the Maillard reaction is generally accompanied by a fall in pH, which may be slowed by the use of buffers (Saunders and Jervis 1966; Spark 1969). Buffers may also act as general acid-base catalysts (Saunders and Jervis 1966). However, the accelerating effect of buffers at acid pH, where the pH change during reaction was small was unexplained. Potman and van Wijk (1989) have concluded that the effect is optimum in the pH range pH 5 to 7 and that the dihydrogen phosphate ion abstracts a proton during the Amadori rearrangement, thus accelerating conversion of the N-glycosylamine to the Amadori product by general base catalysis.

1.1.2.4 Sugars

It has been demonstrated that the aromas generated by heating a range of different amino acids with selected sugars are characteristic of the amino acid, while the rate of reaction depends largely on the sugar (Kiely *et al* 1960; Lane and Nursten 1983). Spark (1969) examined the development of browning of various sugars when heated in aqueous solution at 50 ^{o}C with glycine and showed that they give different rates of browning, such that: pentose sugar (xylose) > aldohexoses (glucose, mannose, galactose) > ketohexoses (fructose, sorbose) > reducing disaccharides (maltose). Among the pentose sugars, ribose gives more rapid browning than xylose and arabinose (Pomeranz *et al* 1962). A similar order of reactivity was obtained by Lewis and Lea (1950), using loss of amino-N as a measure of rate of reaction in systems with a low water content at 25 ^{o}C. Their results differed only in that the reducing disaccharide (maltose) reacted slightly faster than the ketohexose; however, this disparity may be accounted for by the differences in conditions and parameter measured, as neither fructose nor disaccharides developed brown colour under these conditions. Various workers have found that the order of reactivity of sugars depends on the method used to monitor progress of reaction. El'Ode *et al* (1966) obtained different results depending on whether browning or weight of dinitrophenylhydrazine derivatives of carbonyl products was measured, while Shaw and Berry (1977) found that colour development in three hexose amino acid reactions did not parallel the formation of volatile, ether soluble products.

1.1.3 MODEL SYSTEMS CONTAINING GLYCINE OR CYSTEINE

1.1.3.1 Glycine-containing Maillard reactions

The coloured polymeric products of model systems containing glycine and a reducing sugar have been the subject of a number of studies (Hashiba 1986; Nursten and O'Reilly 1986), while others have been concerned with the formation of mutagenic compounds (Nyhammar *et al* 1986; Shinohara *et al* 1986). However, there have been few reports of the volatile products of such systems. El'Ode

et al (1966) reacted glycine with each of several sugars under aqueous conditions, varying in pH and temperature, and monitored the aromas generated and the formation of selected carbonyl compounds. The identities of these compounds depended on the sugar involved and most were also detected in systems containing the more complex amino acids. The formation of pyrazines in the reaction between fructose and glycine has also been studied (Dawes and Edwards 1966). More recently, Hayase and Kato (1986) analysed selected volatile compounds produced by a glucose + glycine system over varying periods of time and pH values. Some pyrroles derived from a glycine-glucose model systems are described by Olsson *et al* (1981). The aromas generated by the glycine + sugar Maillard system have been reported by Kiely *et al* (1960) and Lane and Nursten (1983) who described them to be "caramel-like" and "burnt".

1.1.3.2 Cysteine-containing Maillard reactions

At high temperatures, cysteine itself will break down; volatile compounds obtained after heating at 300 ^{o}C have been found to include a thiazole, a thiazoline, a thiazolidine and several thiophenes (Kato *et al* 1973a) as well as ethylamine, mercaptoethylamine, acetaldehyde, NH_3 and H_2S (Fujimaki *et al* 1969). The aroma of cysteine heated at 200 to 400 ^{o}C was described as "popcorn, sesame-like" but no odour was detected after heating at only 150 ^{o}C (Kato *et al* 1973a). More recently the thermal degradation of cysteine in aqueous solution at 160 ^{o}C was examined and additional compounds identified included di- and trithiolanes, tri- and tetrathianes as well as thiophenethiols and 1,2,3-trithia-5-cycloheptene (Shu *et al* 1985a). The thermal degradation of the dimeric form of this amino acid, cystine, yields many of the same products but also a series of di-, tri-, and tetrasulphides and a greater range of thiazoles (Shu *et al* 1985b). Boelens *et al* (1975) reacted together the primary degradation products of cysteine (acetaldehyde, NH_3 and H_2S) and identified a range of volatile products including oxazoles, thiazoles, trithianes, trithiolanes and dithiazanes as well as bis-(1-mercaptoethyl) sulphide, considered to be a key intermediate in the formation of these compounds.

Early investigations of the sulphur and carbonyl compounds formed in a model Maillard systems containing cysteine and glucose showed the presence of propanal and several simple thiols (Arroyo and Lillard 1970). Mulders (1973) reacted cysteine (and cystine) with ribose under low water conditions and discussed the mechanism of formation of many of the 40 heterocyclic compounds identified. The reaction of cysteine with glucose has been studied by Kato *et al* (1973b) who investigated the effect of temperature on the aromas produced under dry conditions and identified 24 volatile products of this reaction. A number of interesting volatile compounds have also been isolated and identified after heating cysteine with glucose (Scanlan *et al* 1973) or xylose (Mussinan and Katz 1973) under aqueous conditions. These various workers have identified a range of compounds, including thiophenes and thiophenones, furans, pyrazines, thiazoles, sulphides, disulphides and carbonyl compounds. The volatile compounds detected in these cysteine-containing Maillard systems have been listed by MacLeod and Seyyedain-Ardebili (1981).

More recently, pyrazines, oxazoles and thiazoles with various combinations of alkyl substituents have been reported among the volatiles from the reaction between cysteine, cystine or methionine and 2,3-butanedione (Hartman and Ho 1984) and a novel compound, 3-methyl-1,2-dithian-4-one, identified among the volatiles of a model system containing cystine, glutamate, ascorbic acid and thiamin (Hartman *et al* 1984b). The effect of different water activities on the volatile products of this system has been investigated (Hartman *et al* 1984b,c); a greater number and amount of sulphur containing products were detected in the high moisture systems ($a_w > 0.4$) and it is suggested that the higher water activity favours release of H_2S (Hartman *et al* 1984b).

Sheldon *et al* (1986) studied the rate of production of several volatile products resulting from the irradiation of cysteine and ribose at room temperature and found that similar compounds were generated as in a heated system. Shu *et al* (1989) have identified numerous compounds from the reaction between cystine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone, of which 3,5-dimethyl-1,2,4-trithiolanes and thiophenes were the major heterocyclic products. In a comparison between different reaction conditions trithiolanes, thiophenones, furanones and thianes were favoured in

aqueous solution, while more thiazoles and pyrazines were detected on heating in glycerol. Various thiolanones and thianones have been identified as characteristic products of Maillard systems containing cysteine with xylose or glucose (Tressl *et al* 1989).

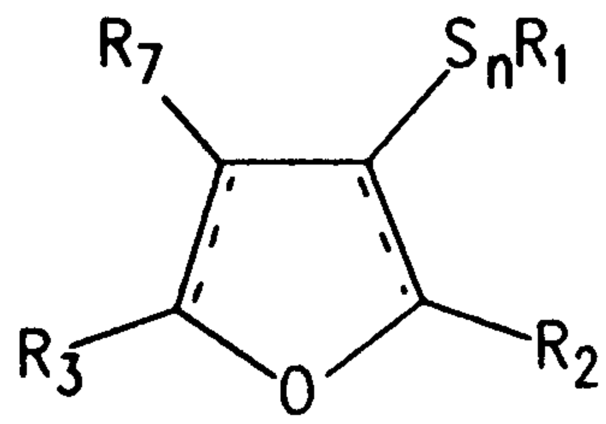
Much of the interest in cysteine-containing Maillard systems has arisen as a consequence of the meat-like aromas formed during this reaction. Early work (Kiely *et al* 1960; Morton *et al* 1960) suggested that reactions between cysteine and various sugars generated compounds with odours reminiscent of cooked meat. Interestingly, while Scanlan *et al* (1973) described the odour of a heated aqueous cysteine + glucose mixture as reminiscent of stewed chicken, the same mixture heated under dry conditions gave an aroma described as "Japanese rice cracker, sesame-like" (Kato *et al* 1973b) or "puffed wheat, Sugar puffs" (Lane and Nursten 1983).

Investigations of the volatile products from such reactions have proved of great interest for the formulation of meat-like flavourings and have resulted in a considerable number of patent applications, especially in the late 1960s and early 1970s. MacLeod and Seyyedain-Ardebili (1981) have summarized eighty GB and US patents based on reactions between meat flavour precursors; most of these include a source of cysteine and a reducing sugar. More recent patents have concentrated on the role of specific compounds in meat-like flavourings; various classes of compounds have been claimed to confer or enhance meat flavour, including various heterocyclic thioethers (van den Bosch 1977, 1979), mercapto-substituted oxathiolanes and oxathianes (Parliment 1985), dimethylheptadienyl-1,3-dithiolanes and methyl(methylthioalkyl)-1,3-dithiolanes (Pittet *et al* 1984a,b). Nearly eighty compounds reported by the literature to possess meat-like aromas have been compiled by MacLeod (1986).

Two patents have described the use of heterocyclic thiols and disulphides of the general structures shown in Figure 1.1U as meat flavourings (Evers 1971; van den Ouweland and Peer 1972). The meat-like odour properties and low odour thresholds of these compounds have made them a subject of considerable interest ever since. The meaty character of these compounds depends on the position of the thio group and the degree of unsaturation; furans and thiophenes with a thiol group in the 3-position tend to possess a "meaty" aroma while those with the thiol in the

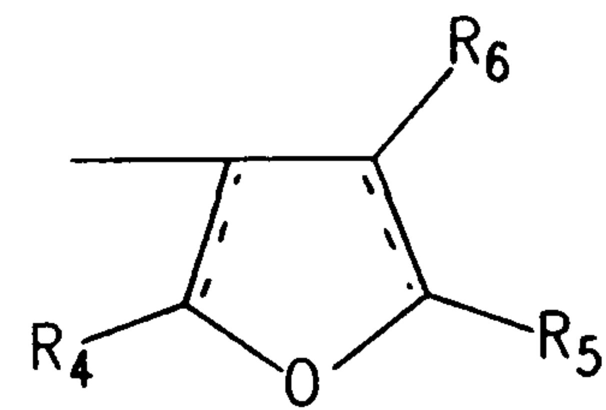
Figure 1.1U: General formulae of some compounds patented for use as meat flavourings

Evers (1971)



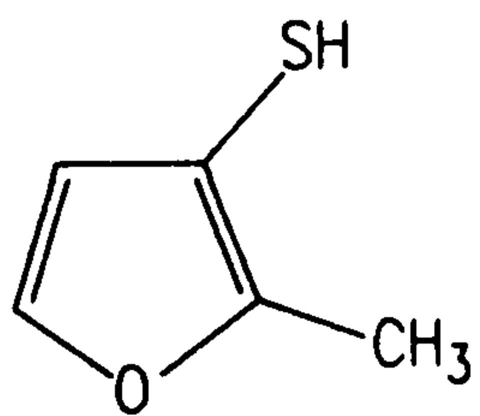
where n = 1 to 4
R_1 = H, alkyl, alkenyl, alkadienyl

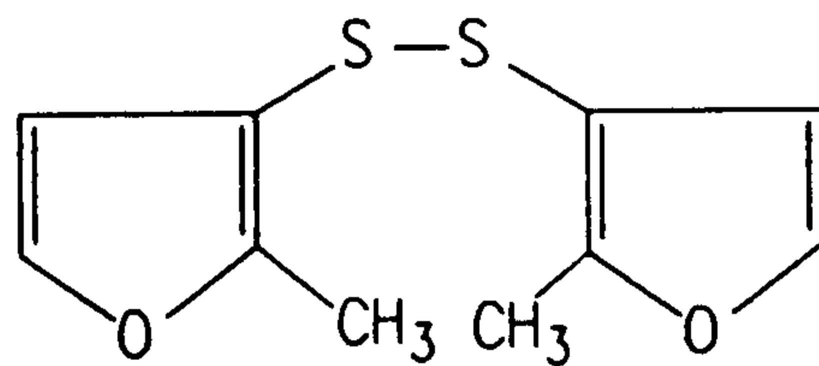
or

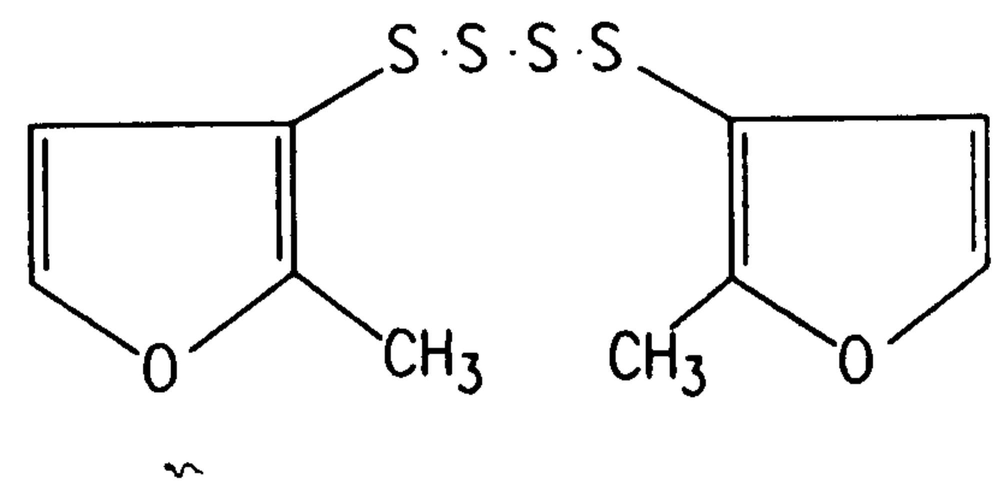


R_{2-7} = H, alkyl, alkenyl, alkadienyl or a cyclic structure

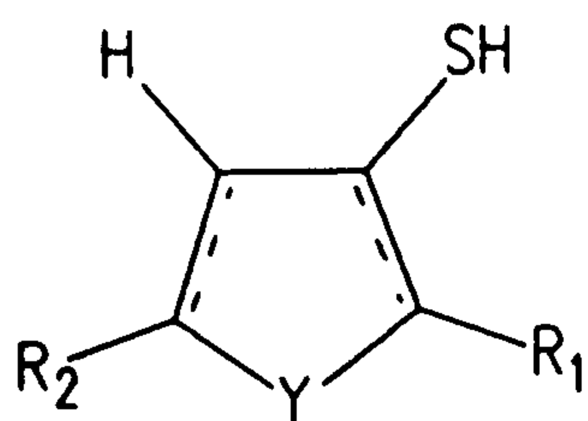
For example:







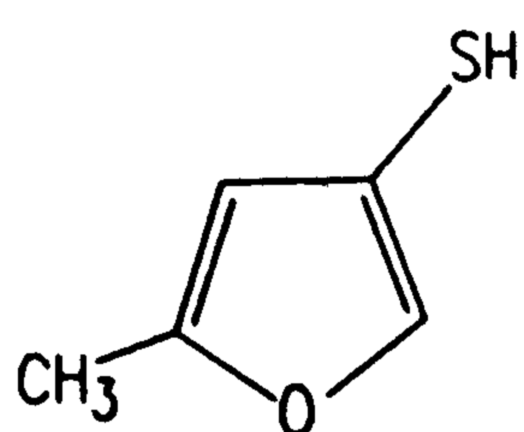
van den Ouweland and Peer (1972)

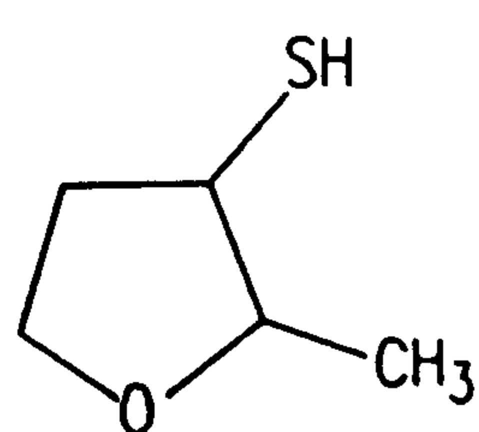


where Y = O or S

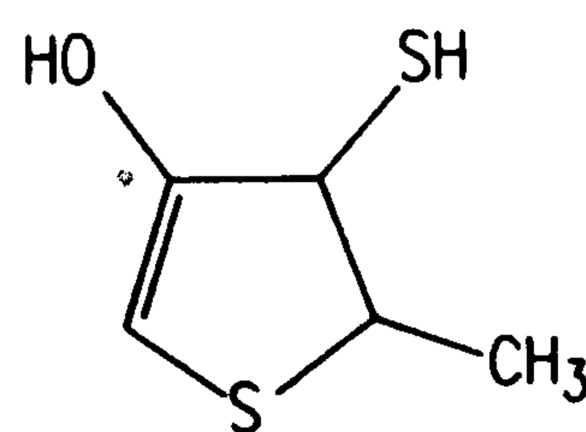
R_1, R_2 = H, methyl, ethyl

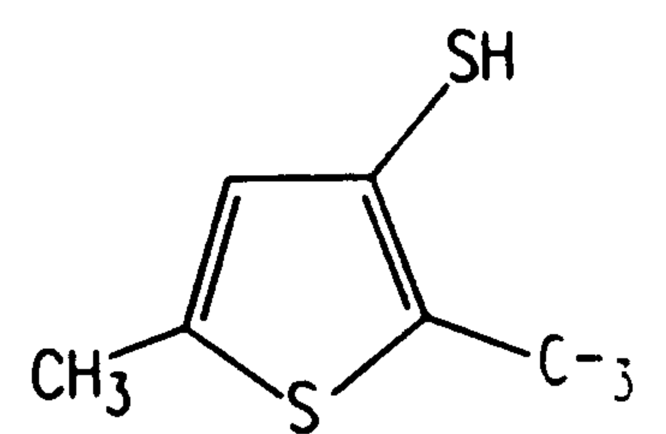
For example:





cis and trans





2-position instead tend to be "burnt" and "sulphurous" (Evers *et al* 1976). The best meat-like aroma is given where there is a methyl group adjacent to the thiol group and the ring contains at least one double bond (van den Ouweland *et al* 1989). Bis (2-methyl-3-furyl) disulphide has one of the lowest odour thresholds known and can be detected at 1 part in 10^{14} (Buttery *et al* 1984).

Recently, members of this class of compounds have also been detected in meat. 2-Methyl-3(methylthio) furan was identified in cooked beef by MacLeod and Ames (1986a,b) and Gasser and Grosch (1988) have used odour dilution studies to show that 2-methyl-3-furanthiol and bis (2-methyl-3-furyl) disulphide are key odour compounds in the aroma of cooked beef. Thus, the formation of these compounds in model systems is of particular interest.

Nineteen related disulphides and thioethers have been detected as volatile products of model systems containing cystine, glutamate, ascorbic acid and thiamin (Werkhoff *et al* 1989, 1990), some of which are also obtained from thiamin alone (Hartman *et al* 1984a; van Dort *et al* 1984). Many of these have been synthesised and their odours described (Werkhoff *et al* 1990); mechanisms of formation have been proposed assuming thiamin to be the primary precursor. However, compounds of this class have also been reported in Maillard systems containing only cysteine, xylose and a general source of amino acids such as hydrolysed soya protein (Misharina *et al* 1987, 1988). Therefore, it seems probable that the monomeric forms are generated by the mechanisms described by van den Ouweland and Peer (1975; Fig. 1.1L) and the disulphides by oxidation of these compounds.

While the complex reactions occurring between various amino acids and sugars and their role in the generation of the characteristic aromas of meat and other foods have been the subject of numerous investigations (as described), few workers have considered the influence of other principal components of foods, such as lipids, on these reactions. The remainder of this Introduction describes the mechanisms of lipid degradation and assesses the available information on interactions occurring between these two major pathways.

1.2 LIPIDS AND FLAVOUR

Lipids, proteins and carbohydrates are major components of most foods. However, unlike proteins and carbohydrates which are largely hydrophilic, lipid is hydrophobic; this characteristic physical property affects the manner in which lipid contributes to the eating quality of foods.

The lipid composition of foods affects not only their flavour but also texture, mouthfeel etc. In part, this is due to the ability of the hydrophobic lipids to form emulsions, which gives milk, cream and other dairy products their characteristic textures. Emulsions of protein, lipid and water are also important in the formation and texture of many restructured meat products, such as sausages. The fat content of meat is believed to affect its juiciness; meats low in fat may seem juicy at first as the juices are released, but then become dry. It is thought that lipids stimulate salivation to give an impression of sustained juiciness (Lawrie 1985).

The flavour and aroma of foods are affected by both the physical characteristics of lipids and their chemical reactivity; the means by which lipid influences these attributes can be categorised as follows:

a Lipids modify the flavour of other components of the food by affecting their transfer to the olfactory receptors. For instance, taste compounds must exist in aqueous solution for detection by receptors on the tongue; lipids can retard the passage of these compounds into the saliva and therefore diminish their effect. Similarly, the odour threshold of an aroma compound can differ markedly between lipidic and aqueous solutions, according to the relative solubilities of the compound in these media (Forss 1972). Fats can also act as a reservoir for lipid-soluble aroma or taint compounds.

b The low volatility of most lipids ensures that they are largely odourless, while their low solubility in water tends to render them tasteless. Fatty acids above C_{10} do not have much flavour, but lower molecular weight homologues can

contribute to rancid odours (eg. butyric acid in milk) and fatty acids below C_4 possess a sour taste (Forss 1972).

c The most important contribution made by lipids to flavour is by virtue of their degradation products. The oxidation of lipids yields a wide range of volatile compounds which contribute to the odours of both heated and unheated foods. At ambient or subambient temperatures, the degradation of lipids causes rancidity and off-flavours in many foods. However, during cooking, similar oxidative pathways yield compounds which contribute to the desirable aroma characteristics of the food in question.

This Section will concentrate on this third aspect of 'lipids and flavour', i.e. the formation of volatile compounds by lipid degradation reactions. The mechanisms of lipid autoxidation are summarized briefly (Sec 1.2.1), followed by an assessment of how these reactions are modified by the application of heat (Sec 1.2.2). The response of phospholipids to oxidation will be evaluated in Section 1.2.3, while Section 1.2.4 examines the groups of compounds most commonly produced by the degradation of lipid.

1.2.1 AUTOXIDATION OF LIPIDS

The reaction of lipids with molecular oxygen is, theoretically, forbidden due to the difference in spin states between these two species (Kanner *et al* 1987). The ground state of unsaturated fatty acids corresponds to the singlet state (with two paired electrons in the outer electron shell), while ground state oxygen is in the triplet state (with two unpaired electrons of the same spin but in different orbitals). To overcome the spin restriction imposed by these spin states, one or other reactant must be activated. Either the unsaturated lipid may be activated by the formation of a resonance-stabilized radical, as in autoxidation, or the oxygen may be converted to the singlet state, as in photosensitized oxidation (Gunstone 1984). Alternatively, one or other of the reactants may be activated by enzyme action. These three mechanisms yield distinct, although similar, products. In cooked

meat and in heated model reactions, lipid oxidation occurs almost entirely by free radical mechanisms; photosensitized and enzyme catalyzed oxidation play little part in these systems.

The mechanisms and products of lipid autoxidation have been studied extensively and the reader is referred to numerous reviews on the subject (for example: Grosch 1982; Forss 1972; Frankel 1982, 1985; Chan 1987). No attempt is made to review all the original literature on this subject; instead, this Section outlines briefly the basic mechanisms of autoxidation, concentrating on those aspects of the subject of particular relevance to the studies described later in this Thesis.

The autoxidation of lipids at ambient and subambient temperatures proceeds via a free radical chain reaction and any one fatty acid may oxidize to give a number of different volatile products. Naturally occurring fats are composed of a complex mixture of fatty acids and their oxidation yields a wide range of volatile compounds including alcohols, aldehydes, ketones, alkanes, alkenes, acids and esters.

Lipid autoxidation reactions proceed in three stages:

1. Free radical oxidation of fatty acids to form hydroperoxides.

2. Breakdown of hydroperoxides to give a range of volatile oxidation products.

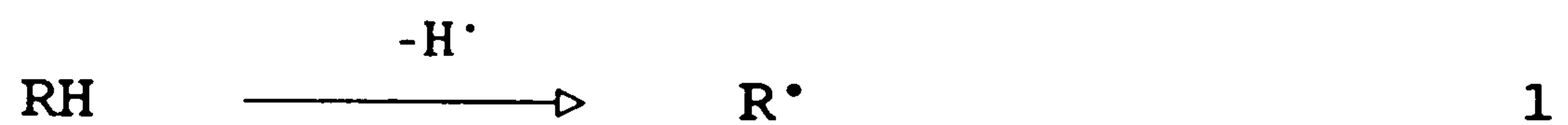
3. Secondary oxidation reactions of hydroperoxides and their breakdown products.

1.2.1.1 Formation of hydroperoxides

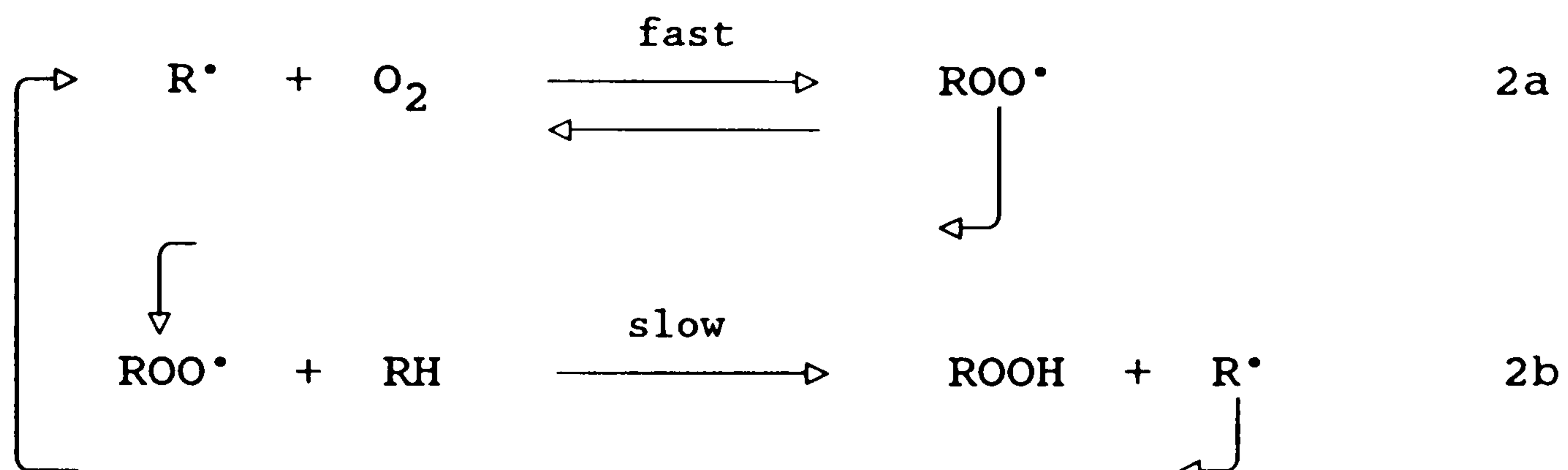
The steps involved in the free radical breakdown of olefinic and polyolefinic substances were suggested by Farmer *et al* in 1942, and these are still recognised as forming the basic pathways of lipid autoxidation (Grosch 1982). The main initiation, propagation, branching and termination steps for the autoxidation of lipids are illustrated in Figure 1.2A.

Figure 1.2A: Free radical mechanism of lipid autoxidation (Grosch 1982; Frankel 1985)

Initiation

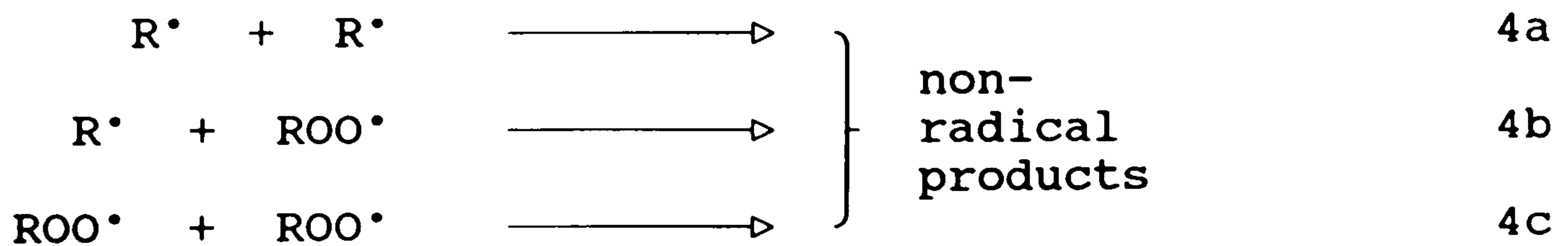


Propagation



Branching

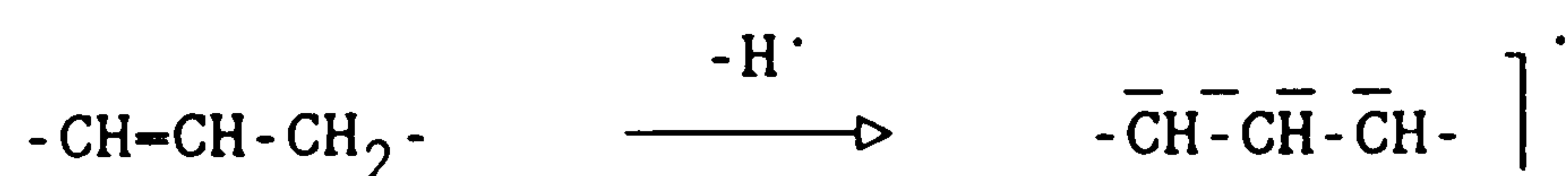
Termination



Initiation (step 1, Figure 1.2A)) involves the formation of free radicals by the abstraction of a labile hydrogen atom from a fatty acid methylene group to give a lipid radical. This can be induced by thermal or photodecomposition of already existing peroxides or hydroperoxides, by metal catalysis, ionizing radiation or by uv irradiation (Schaich 1980a; Frankel 1985). However, the origin of the first radicals to occur in any system is still open to question; it is thought that they may arise from photosensitized oxidation (Grosch 1982), from reaction with transition metals (Ingold 1962; Love 1985) or even from atmospheric pollutants (Pryor and Prier 1980).

Propagation reactions take over from those responsible for initiation once the first hydroperoxides are generated, and promote the continuation of autoxidation by repeatedly converting lipid radicals into hydroperoxides and creating more lipid radicals in the process. Step 2a (Figure 1.2A) involves the incorporation into the lipid radical of a molecule of O_2 to give a peroxy radical, while in step 2b an H atom is abstracted from a further unsaturated fatty acid or lipid to give the hydroperoxide and a lipid radical R˙. In the presence of oxygen, reaction 2a is extremely fast, while 2b is usually rate limiting in the autoxidation process (Uri 1961). Because step 2b is slow, hydrogen abstraction by the species ROO˙ from an unsaturated fatty acid (RH) is selective for the most weakly bound hydrogen (Frankel 1985).

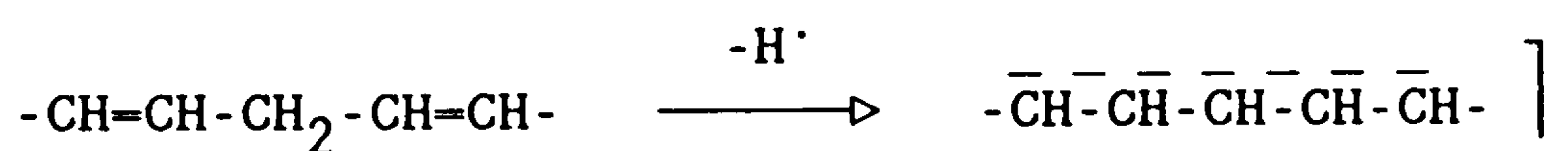
The factors dictating the site of abstraction of the H radical and the subsequent O_2 addition also determine which hydroperoxides are generated and, therefore, which volatile products will be formed. In an unsaturated fatty acid, the H atoms most labile to attack by ROO˙ (step 2b) are those of methylene groups adjacent to a double bond, due to stabilization of the resulting free radical by the formation of a resonance hybrid:



A methylene group adjacent to two double bonds in a polyunsaturated fatty acid is particularly vulnerable to abstraction of an H atom, as the resulting radical is further stabilized by conjugation with both double bonds:

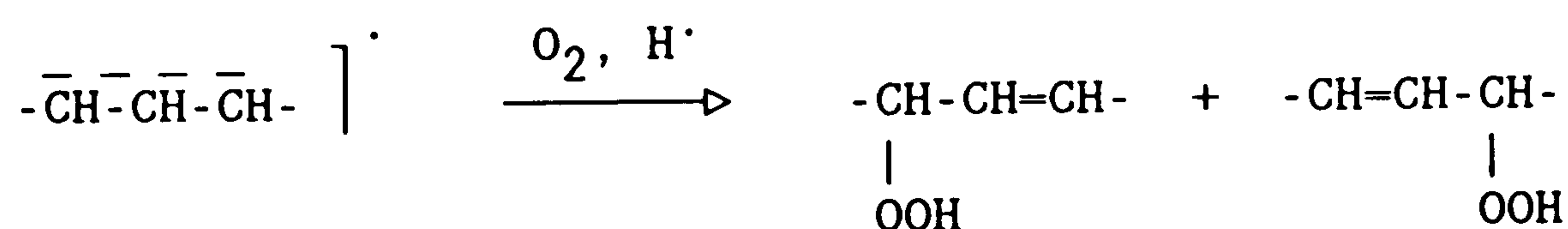
Table 1.2a : Dissociation energy of different classes of hydrogen atom (Bolland 1949; Szwarc 1950).

Position of H atom	Dissociation energy D_{R-H} (kcal/mole)
R-CH₂ with H on CH₂ ($R-CH_2-H$)	95-101
$R-CH(H)-CH_3$	ca.89
$R-CH=CH(H)$	ca.104
$R-CH=CH-CH(H)-R'$	76-85
$R-CH=CH-CH(H)-CH=CH-R'$	68

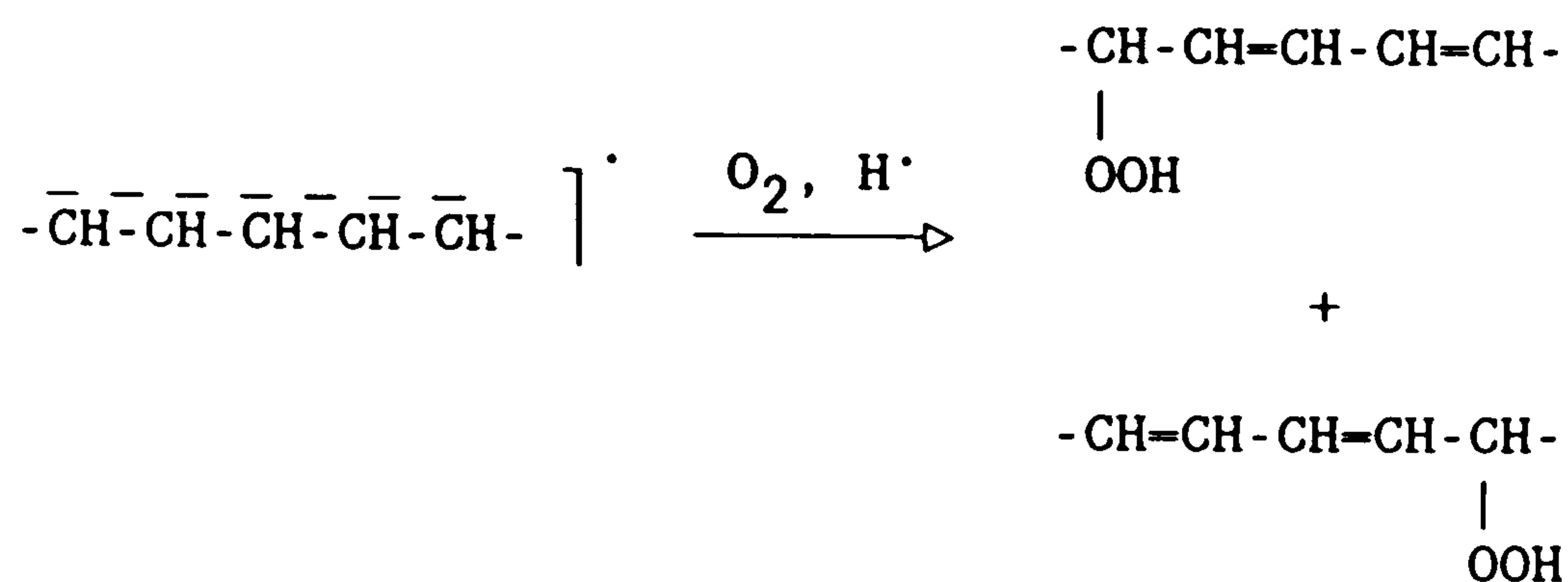


The ease of abstraction of different classes of H atom can be assessed from the bond dissociation energies cited in Table 1.2a.

Oxygen addition to a lipid free radical occurs at one or other end of the resonating system, giving the maximum degree of conjugation.



Where the resonating system is a 1,4-pentadiene system, it might have been expected that three hydroperoxides would be formed, with O_2 attack at the middle as well as the ends of the system. However, it was shown by Sephton and Sutton (1956), in studies on linoleic acid, that the only hydroperoxides detectable arise from O_2 addition at each end of the system.



The expected hydroperoxides from oleic, linoleic, linolenic and arachidonic acid can be predicted according to this principle, as shown in Figures 1.2B,C,D and E. It may be predicted that four hydroperoxides will be formed from oleic acid and linolenic acid. Only two hydroperoxides would be expected by hydrogen abstraction from C_{11} of linoleic acid, although theoretically others could be formed by hydrogen abstraction at C_8 or C_{13} (MacLeod and Ames 1988). Both Grosch (1982) and Frankel (1985) have reviewed the available data on the yield of hydroperoxides from the autoxidation of oleate, linoleate and linolenate, and have concluded that the hydroperoxides formed are those predicted in Figure 1.2B-D, but that the isomeric proportions are not always even (Table 1.2b).

The four double bonds of arachidonic acid provide three sites for hydrogen abstraction, generating three pentadienyl radicals and six hydroperoxides (Fig. 1.2E and Table 1.2b; Terao and Matsushita

Table 1.2b : Isomeric hydroperoxides from the autoxidation of methyl esters (Frankel 1985; Terao and Matsushita 1981).

	Isomeric distribution (relative %)					
Oleate	8-OOH 26-28	9-OOH 22-25	10-OOH 22-24	11-OOH 26-28		
Linoleate		9-OOH 48-53	13-OOH 48-53			
Linolenate	9-OOH 28-35	12-OOH 8-13	13-OOH 10-13	16-OOH 41-52		
Arachidonate*	5-OOH 18	8-OOH 10	9-OOH 16	11-OOH 13	12-OOH 9	15-OOH 34

* Hydroperoxides estimated as the monohydroxy breakdown products

Figure 1.2B Hydroperoxide formation in oleic acid

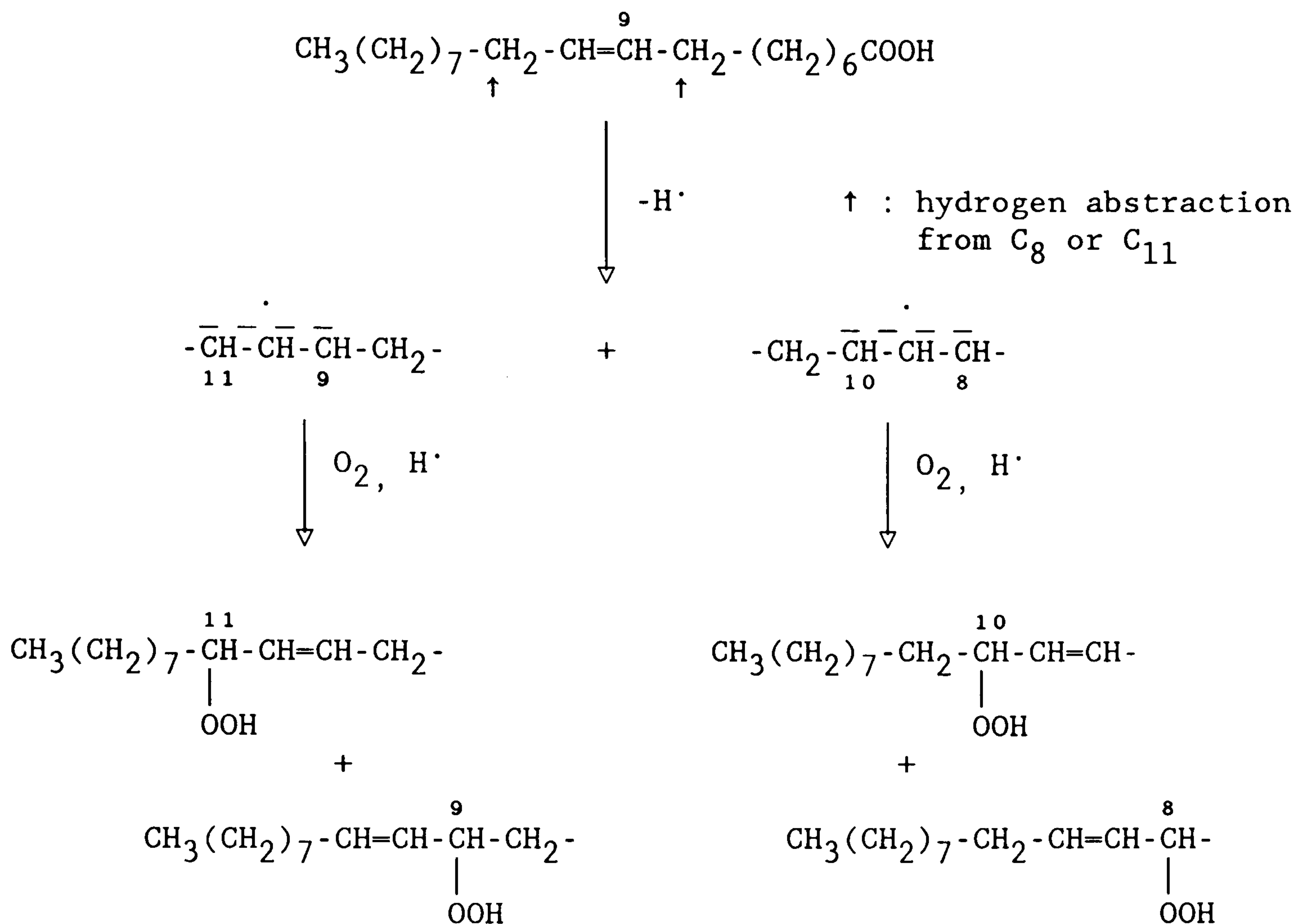


Figure 1.2C Hydroperoxide formation in linoleic acid

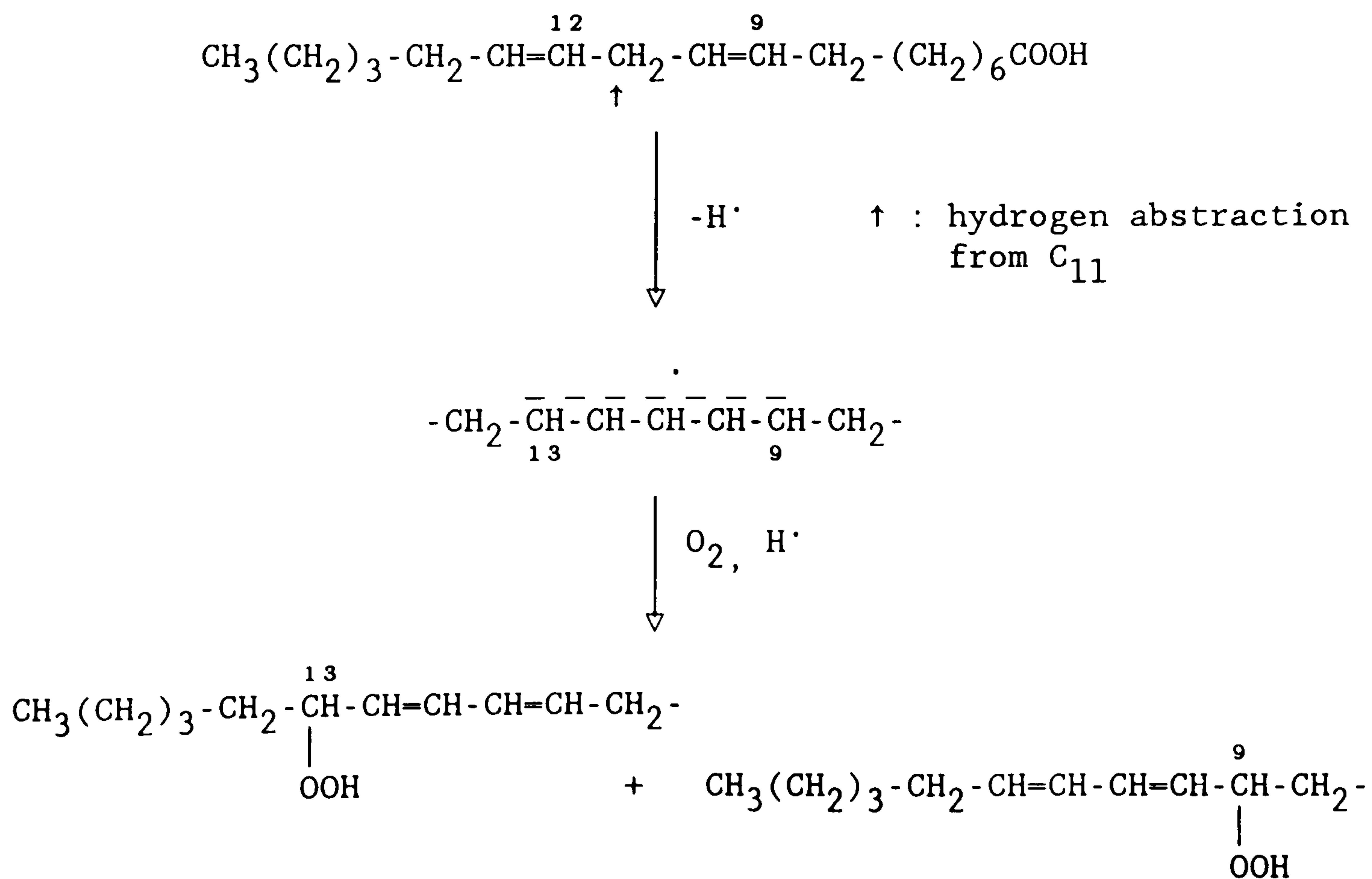


Figure 1.2D Hydroperoxide formation in linolenic acid

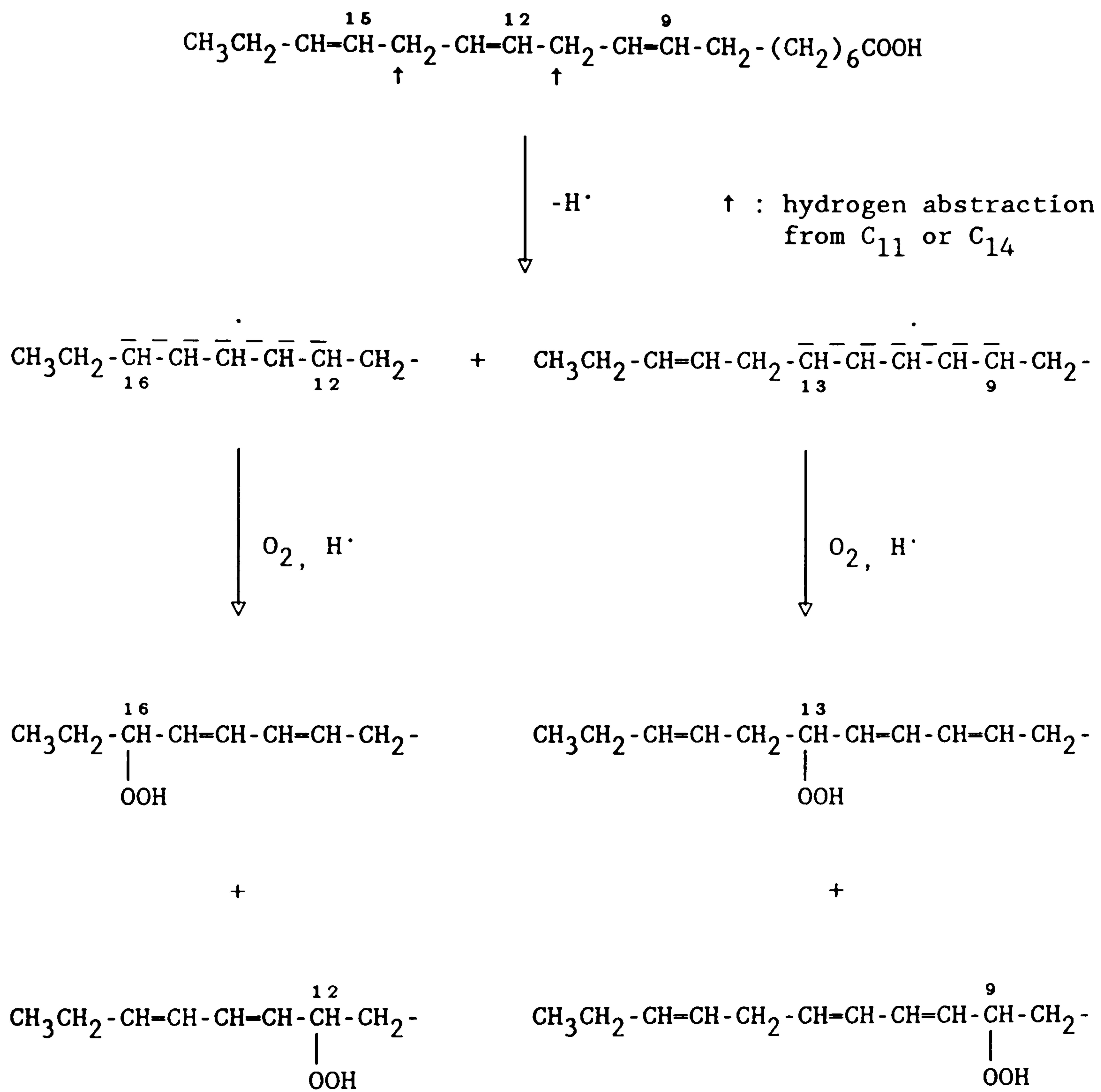
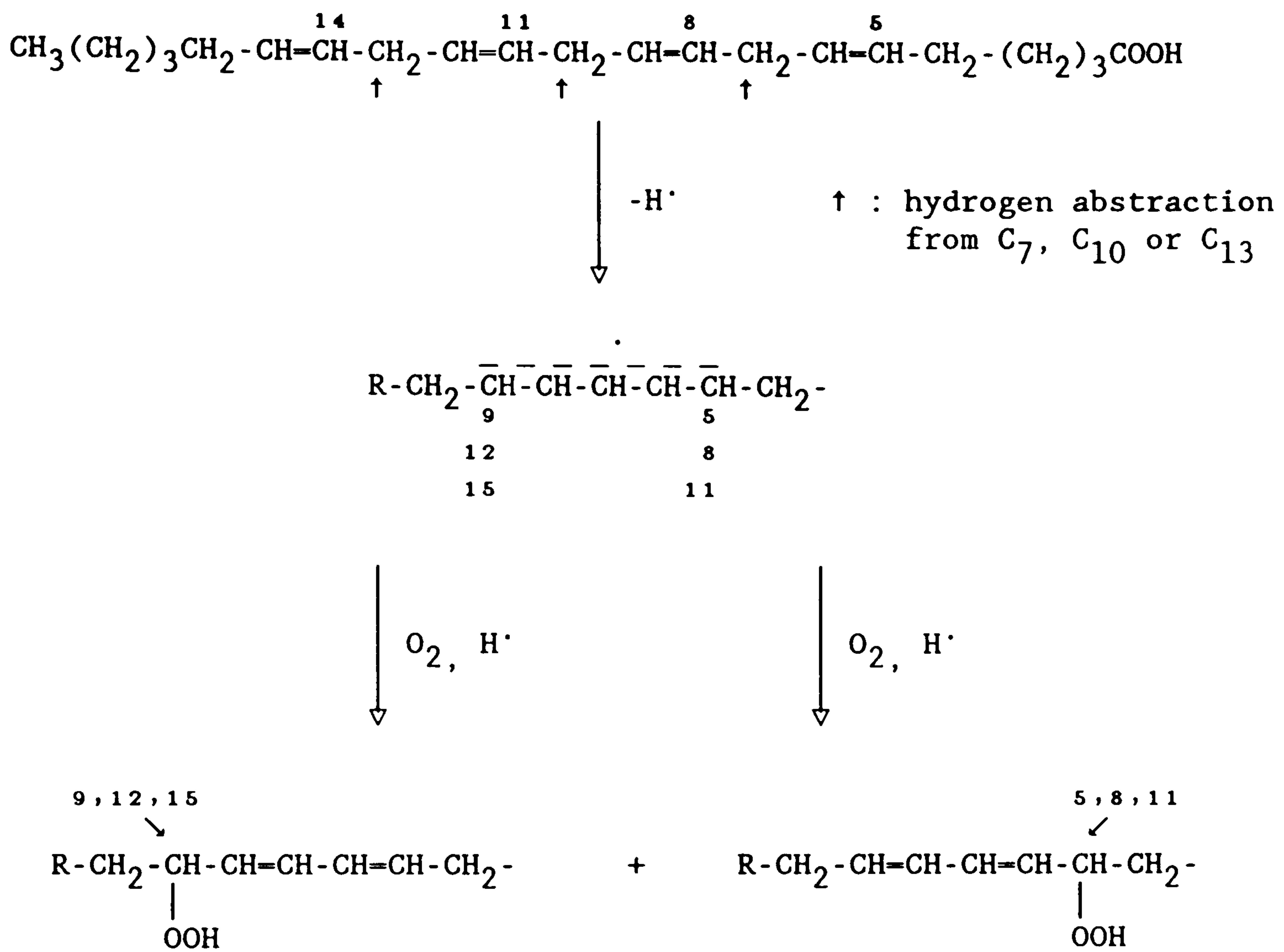


Figure 1.2E Hydroperoxide formation in arachidonic acid



For 9- and 5-hydroperoxides, R = $CH_3(CH_2)_3CH_2$-CH=CH-CH_2-CH=CH-

For 12- and 8-hydroperoxides, R = $CH_3(CH_2)_3CH_2$-CH=CH-

For 15- and 11- hydroperoxides, R = $CH_3(CH_2)_3$-

1981). The autoxidation of eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids generate eight and ten hydroperoxides respectively (Yamauchi *et al* 1983; Van Rollins and Murphy 1984). The tendency of linolenic acid to favour the production of the two extreme hydroperoxides was originally thought to be due to rapid secondary oxidation and/or migration of the OOH group (Frankel 1961, Chan *et al* 1977); however, it appears that this phenomenon is caused by 1,3-cyclization of the 12- and 13-hydroperoxides to give hydroperoxy cyclic peroxides as suggested by Haverkamp Begemann *et al* (1968) and Frankel (1985). Figure 1.2F illustrates how such a mechanism can cause cyclization of the 12- but not the 16-hydroperoxide from linoleate; a similar comparison could be made between the 13- and 9-hydroperoxides.

The **branching** reactions (shown in steps 3a and 3b in Fig. 1.2A) cause an increase in the rate on reaction with time, known as autocatalysis. Early in the autoxidation process, step 3a is the major branching mechanism and yields alkoxy radicals which can further propagate the reaction by step 2c. When the amounts of hydroperoxide have increased to a certain level, the bimolecular reaction (3b) is favoured (Grosch 1982).

Termination of free radical autoxidation occurs by the reaction of two radicals (steps 4a, 4b and 4c). Except in circumstances where the levels of O_2 are limiting, the fast rate of reaction of step 2a ensures that all R˙ is converted to ROO˙; termination then occurs only by step 4c (Uri 1961). However, the concentration of O_2 may be low enough in the inner layers of a fat or food for reactions 4a and 4b to occur (Grosch 1982).

Factors affecting the rate of oxidation

The rate of oxidation is affected by many factors, including degree of unsaturation of the fatty acids and oxygen availability; the concentration and amount of oxygen available will affect the rate and extent of oxidation, and may also determine the favoured free radical mechanisms, as mentioned earlier. However, the rate of autoxidation of lipids is affected not only by the lipid and oxygen themselves, but also by other components of the system.

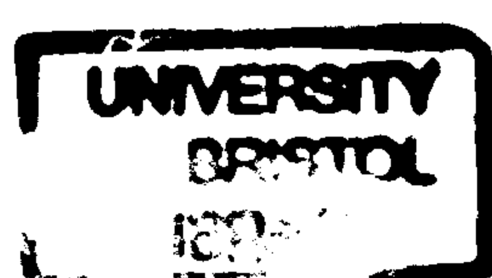

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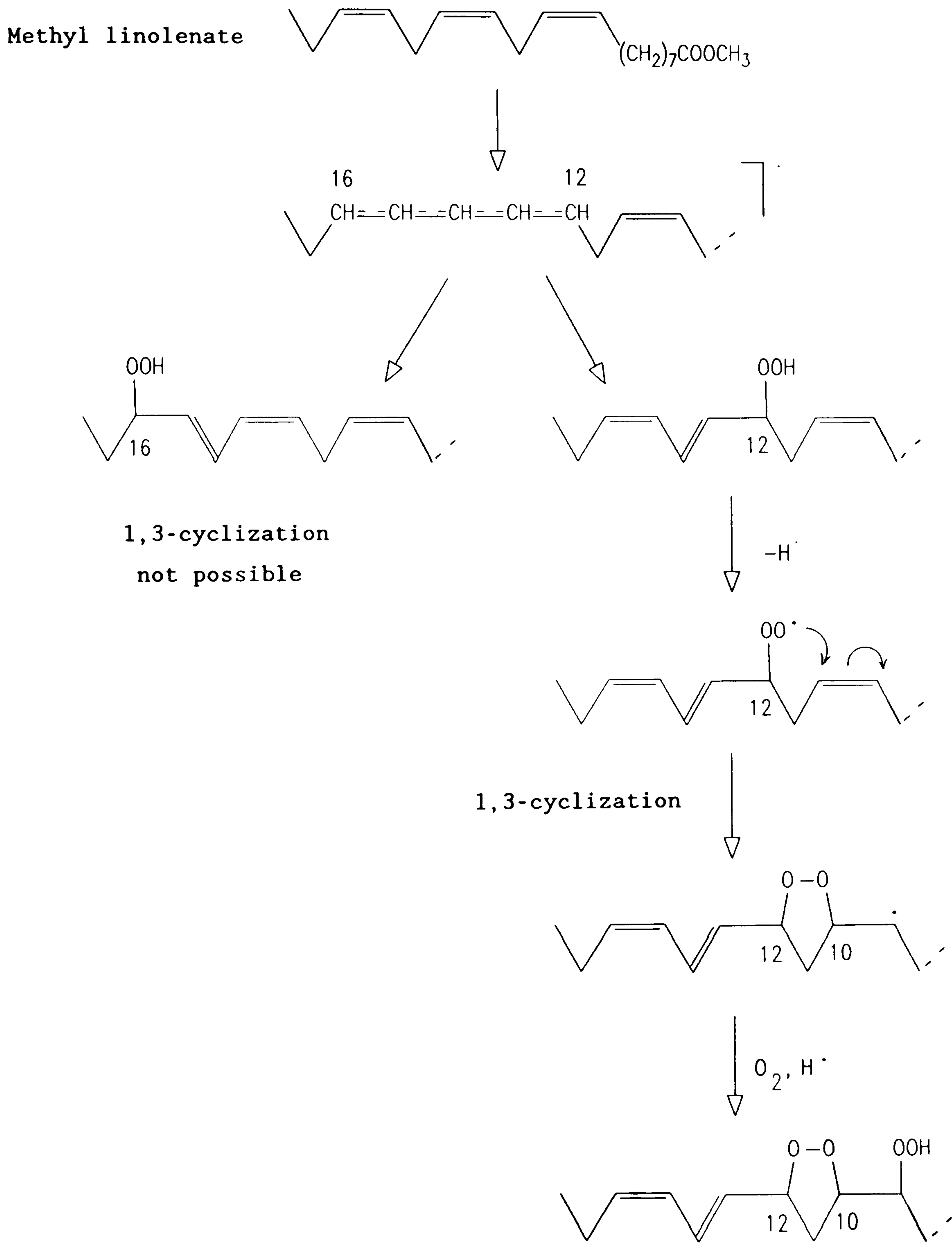
Figure 1.2F: 1,3-cyclization of the 13- (but not the 16-) hydroperoxide of linolenate (Haverkamp Begemann 1968; Frankel 1985).



Where the lipid forms part of a food, a wide variety of pro- and antioxidants may be present. Pro-oxidants include transition metal ions, haem compounds, certain enzymes and light, while antioxidants include a variety of natural and synthetic compounds capable of inhibiting the free radical reaction. The effect of each of these factors is considered in brief.

a) Fatty acid composition

The relative reactivities of a number of fatty acids to autoxidation have been assessed in terms of their induction period (Privett and Blank 1962), oxygen uptake (Stirton *et al* 1945; Holman and Elmer 1947; Lea 1952) and peroxide development (Gunstone and Hilditch 1945); although these studies were conducted under a variety of experimental conditions, the results summarized in Table 1.2c enable a rough comparison of the rates of oxidation of the more common fatty acids.

The much increased reactivity of linoleate over oleate is due to the additional double bond and the consequent reduction in C-H dissociation energy. The addition of a further double bond in linolenate creates two doubly allylic methylene groups, which, in

Table 1.2c : The relative reactivities of various fatty acids to autoxidation.

Parameter measured	Fatty acid					Reference
	18:0	18:1	18:2	18:3	20:4	
Peroxide development	-	1	12	ca25	-	Gunstone and Hilditch 1945
Oxygen uptake	0.25	1	51	96	-	Stirton *et al* 1945
" "	-	1	41	98	195	Holman and Elmer 1947
" "	-	1	20	50	-	Lea 1952
Induction period (h)	-	82.0	19.0	1.34	1.0	Privett and Blank 1962

turn, cause a two-fold increase in the rate of oxidation. The bond energy itself is not altered as neither of the possible radicals is stabilized by conjugation to the more distant vinyl group (Frankel 1985).

The formation of hydroperoxides from pure saturated fatty esters at 60 ^{o}C or below is negligible, due to the extremely slow rate of initiation. However, the presence of small amounts of an unsaturated fatty ester can promote autoxidation of the saturated fatty esters (Brodnitz *et al* 1968). This suggests that insufficient energy is available for initiation in saturated fatty acids themselves, due to the high C-H bond dissociation energy; however, if another more reactive species is present to initiate the reaction, propagation reactions do occur. Therefore, in natural lipid systems, containing a mixture of fatty acids, the autoxidation of saturated fatty acid moieties will contribute to the formation of volatile products, albeit to a lesser extent than the unsaturated fatty acids (Brodnitz 1968).

Natural fats and oils, such as those present in most foods, contain a complex mixture of fatty acids, ranging from saturated to highly unsaturated. From the reactivities shown in Table 1.2c, it might be expected that the oxidation products of such a mixture would be formed almost entirely from the polyunsaturated fatty acids present. Some workers have indeed found the ratio of the different hydroperoxides from a mixture of oleate, linoleate and linolenate to be consistent with their relative rates of oxidation (Stirton *et al* 1945; Fatemi and Hammond 1980). However, other studies have indicated that the relative contributions from different fatty acids may be confounded by interactions between them. It has been shown that the autoxidation of oleate can be greatly accelerated by the presence of linoleate (Gunstone and Hilditch 1945; Romero and Morton 1975). The pro-oxidant effect of linoleic acid is greatest when it is present at less than 25%; at greater than 50% the effect disappears. It is proposed that a small concentration of the more highly reactive linoleic hydroperoxides increases the overall rate of propagation. However, at higher levels of linoleic acid, the amount of these reactive hydroperoxides become such that the rate of termination increases, causing the progressive disappearance of the pro-oxidative effect (Romero and Morton 1975).

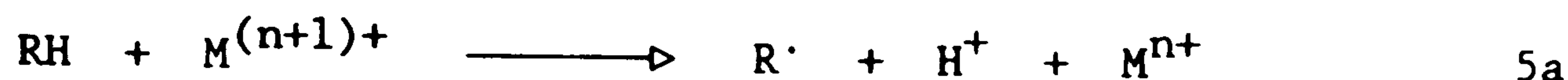
In a 9:1 oleate-linoleate mixture, oxidized at 80°C, 50% of the hydroperoxides were derived from the linoleate; at a later stage of this reaction this value was reduced to <7% (Frankel *et al* 1977a). Similar reductions in linoleate peroxides were observed for other ratios of oleate to linoleate. These effects were probably caused by the high relative importance of linoleate oxidation during initiation, and the subsequent loss of hydroperoxides through termination reactions, as described above. In addition, depletion of unoxidized linoleate at the later stages of reaction may have contributed. In similar studies on hydroperoxide formation in oleate-linoleate-linolenate mixtures, the proportion of hydroperoxides from linolenate was lower than might have been expected (Frankel *et al* 1977b). This was thought to be due to the high rate of decomposition of linolenate hydroperoxides by cyclization and secondary oxidation reactions.

b) Transition metals and haem compounds

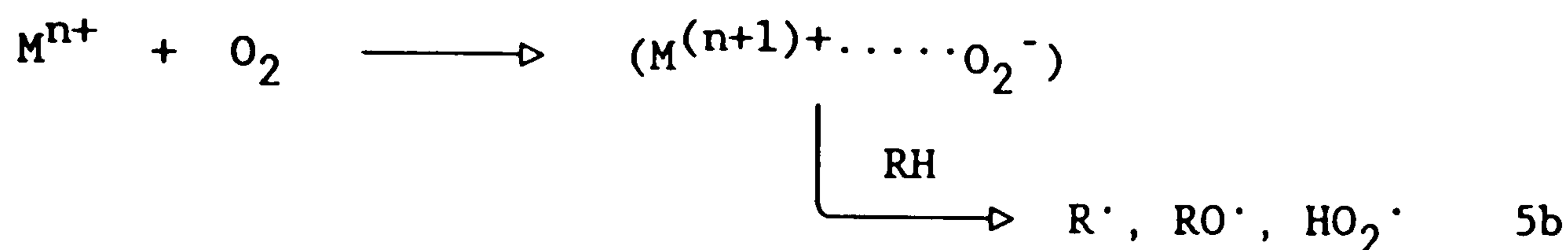
The role of transition metals and haem compounds in the catalysis of autoxidation has been studied extensively and the reader is referred to the relevant sections in reviews by Ingold (1962), Tappel (1962), Labuza (1971), Grosch (1982), Love (1985) and Kanner *et al* (1987).

Transition metals possessing two or more valency states, with a suitable oxidation-reduction potential between them, can promote oxidation both by shortening the induction period and by catalysis of radical forming reactions. Such metals include iron, cobalt, copper, nickel and manganese.

The reduction of the induction period is effected by lowering the activation energy of the initiation step (Labuza 1971). Metals initiate lipid free radical formation most readily in their higher valency state, by a one electron transfer (reaction 5a). The lower valency state may react with dissolved O_2 to give an activated complex (5b), which can undergo a variety of reactions with lipid to generate free radicals (Ingold 1962). Reaction 5a is thought to be the most important initiation reaction in polar solvents (Ingold 1962; Love 1985).

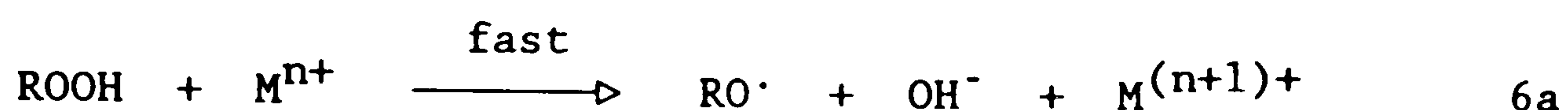


$$RH + M^{(n+1)+} \longrightarrow R^{\cdot} + H^{+} + M^{n+} \quad \text{5a}$$



$$M^{n+} + O_2 \longrightarrow (M^{(n+1)+}\cdots\cdots O_2^{-}) \xrightarrow{RH} R^{\cdot},\ RO^{\cdot},\ HO_2^{\cdot} \quad \text{5b}$$

Transition metals also catalyse autoxidation by direct reaction of either valency state of the metal with hydroperoxides.



$$ROOH + M^{n+} \xrightarrow{\text{fast}} RO^{\cdot} + OH^{-} + M^{(n+1)+} \quad \text{6a}$$

$$ROOH + M^{(n+1)+} \xrightarrow{\text{slow}} ROO^{\cdot} + H^{+} + M^{n+} \quad \text{6b}$$

The lower valency state reacts with hydroperoxide extremely fast, acting as an electron donor, to give the alkoxy radical (reaction 6a). The higher valency state can also react with hydroperoxides (6b), albeit more slowly, giving a peroxy radical and regenerating the original valency state of the metal (Uri 1961). Thus, these reactions represent true catalysis, despite a gradual loss of metal ions due to various complexing reactions (Labuza 1971).

The catalytic effect of transition metals is further complicated by their coordination behaviour in different solvent systems. A polar solvent (water, ethanol etc) tends to decrease the catalytic effect by occupying the coordination shell of the metal and by H-bonding to the hydroperoxides; transition metal catalysis is most effective in non-polar solvents (Uri 1961; Labuza 1971).

Under conditions of low oxygen pressure, transition metals can exert an antioxidative effect. The explanation for this apparently contradictory phenomenon is as follows: the termination reaction is second order with respect to metal catalysts and competes with the initiation reaction, which is only first order with regard to the metal. Thus, at certain concentrations of metal ions and oxygen, the inhibiting effect can become more important than the usual pro-oxidative effect (Marcuse and Fredriksson 1971).

In biological systems, the presence of haem compounds also affects the rate of oxidation. Their role in meat is of particular interest, and this is reflected in the following assessment of the effects of these substances.

Haem compounds include haemoglobin, myoglobin and cytochrome c; in all of these the iron is bound to four nitrogen atoms of a porphyrin ring. Ferric haem compounds seem to be more effective than ferrous forms, while ferrihaemoglobin is more active than ferrimyoglobin with oxidized cytochrome-c having intermediate activity (Grosch 1982; Love 1985). However, in meat, haemoglobin occurs in the red blood cells and myoglobin in the muscle; thus, it may be expected that the myoglobin would have greater contact with lipid-containing membrane structures.

The most important mechanism for the catalysis of autoxidation by haem compounds is the catalytic decomposition of hydroperoxides to give free radicals; the proposed mechanism involves the coordination chemistry of the haem iron. In addition to the four in-plane bonds to porphyrin nitrogen atoms, the iron is thought to bond to two further ligands to give an octahedral structure; these are usually the N of histidine (eg in the myoglobin protein) and a molecule of water (Grosch 1982). It has been proposed that, during catalysis, a hydroperoxide molecule displaces the H_2O in the coordination sphere of the iron and that this results in homolytic cleavage to give an alkoxy and an hydroxyl radical (as in step 3a, Fig. 1.2A). Unlike metal catalysis, a valency change does not seem to be involved (Tappel 1962). Only iron- and mangani-protoporphyrins were found to catalyse the decomposition of hydroperoxides in this way; the inactivity of zinc- and cupric-complexes is explained by their lack of available coordinating orbitals for bonding to the hydroperoxide (Tappel 1962). There is evidence to suggest that the formation of hydrogen peroxide during storage of meat is thought to activate metmyoglobin to a form where its catalytic effect is enhanced (Harel and Kanner 1985).

The role which haem compounds and non-haem iron play in lipid oxidation has been the subject of much investigation, but is still a matter of controversy. While both haem and non-haem iron appear to catalyse lipid oxidation in raw meat (Wills 1966), there is a body of evidence, reviewed by Love (1985), which suggests that the non-haem, rather than the haem iron, is responsible for the rapid

oxidation of lipids in cooked meat. The addition of ferrous or ferric ions to water-extracted muscle, from which much of the myoglobin has been removed, causes acceleration of lipid oxidation after cooking, while added haem compounds appear to have little effect (Sato and Hegarty 1971; Love and Pearson 1974). However, Johns *et al* (1989) suggested that these results could be a consequence of the difficulty of dispersing haem compounds in the meat and found that, in washed muscle and model systems, the pro-oxidative activity of added haemoglobin exceeded that of inorganic iron in both heated and unheated systems. Igene *et al* (1979) found that non-haem iron was released from haem compounds during the cooking of beef; thus the contribution of haem compounds to the oxidation of lipids during cooking may arise from their breakdown and the consequent release of non-haem iron. Rhee (1988) concludes that activated metmyoglobin and enzymes are responsible for the initiation of lipid oxidation in raw meat and that metal catalysts (eg, non-haem iron) promote lipid oxidation via the propagation of free radicals. In cooked meat, haem iron may still initiate lipid oxidation but the catalytic effect of non-haem iron is enhanced.

c) Light

Light can promote lipid oxidation in two ways; by photosensitized oxidation and photolytic autoxidation (Frankel 1985). In the latter mechanism, although uv light promotes the breakdown of peroxides and hydroperoxides, the mechanisms involved are the same free radical reactions as those already described for autoxidation. In contrast, photosensitized oxidation occurs by different pathways and gives a different set of hydroperoxides and products. The mechanism involves the excitation of sensitizer molecules and the transfer of the acquired energy to give activated oxygen molecules (singlet oxygen, $^1O_2$); these react with lipid to create hydroperoxides. Such biological substances as pigments, riboflavin, chlorophyll and haem compounds may act as sensitizer molecules (Hsieh and Kinsella 1989). It is possible that such a mechanism is of particular importance in the formation of the very first hydroperoxides, during initiation (Grosch 1982). Although the formation of hydroperoxides by photosensitized oxidation is by a mechanism quite distinct from that of free radical autoxidation, the hydroperoxides formed decompose to give free radicals and thus promote autoxidation (Hsieh and Kinsella

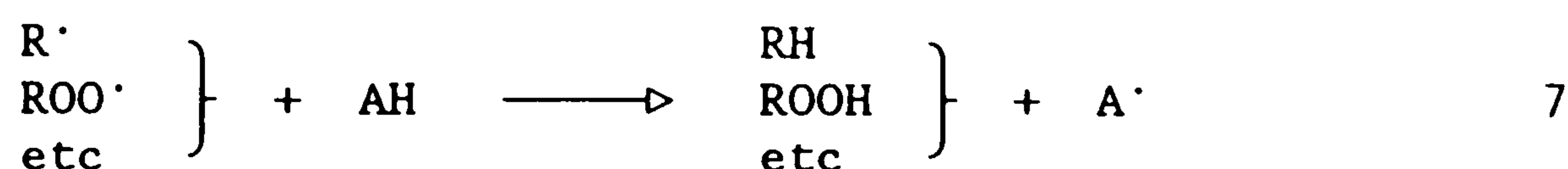
1989). Various species of activated oxygen, including singlet oxygen and hydroxyl radicals, may be formed in biological systems not only by the action of light, but by enzymatic pathways or reaction with transition metal ions (Hsieh and Kinsella 1989).

For further information on photosensitized lipid oxidation the reader is referred to reviews by Schaich (1980b), Grosch (1982) and Frankel (1985).

d) **Antioxidants**

Antioxidants have been classified into three types by Scott (1965) and Labuza (1971), according to their mode of activity. These are as follows:

Type I : 'Free radical chain stoppers'; these are mainly phenolic compounds (represented as AH) which are capable of donating a hydrogen radical to a lipid radical:



$$\left.\begin{array}{l} R^{\cdot} \\ ROO^{\cdot} \\ etc \end{array}\right\} + AH \longrightarrow \left.\begin{array}{l} RH \\ ROOH \\ etc \end{array}\right\} + A^{\cdot} \qquad 7$$

The resulting antioxidant radical is stabilized by resonance and is therefore incapable of generating further free radicals (Sherwin 1985). In effect, such antioxidants promote termination reactions. Although the $A^{\cdot}$ radical does not propagate the free radical reaction (at ambient temperatures), it may react with itself or lipid radicals in further termination reactions (Frankel 1980). Examples commonly used in foods include natural antioxidants such as the tocopherols (including vitamin E), flavonoids (eg quercetin) and synthetic compounds such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene); see reviews by Scott (1965), Labuza (1971) and Houlihan and Ho (1985). These antioxidants generally lose their efficiency at elevated temperatures, as homolytic cleavage of the hydroperoxides generated in reaction 7, and the reaction of AH with O_2, may then generate further free radicals (Frankel 1980).

Type II : 'Free radical production preventors'; these are usually chelating agents which bind metal ions, thus reducing their catalytic activity. Such compounds include EDTA

(ethylenediaminetetraacetic acid), polyphosphates, citric acid and, under dry conditions, ascorbic acid (Labuza 1971). Examples of the use of these antioxidants for the protection of foods have been given by Porter (1980) and Ang and Young (1989).

Type III : 'Environmental factors', including atmospheric conditions, moisture content, exclusion of light etc. Such methods are used commercially, for example, in modified atmosphere packaging, where the oxygen content within the package is reduced and vacuum packaging. Hwang and Regenstein (1988) have compared vacuum packaging with various type I and II antioxidants for the storage of menhaden fish mince and have shown vacuum packaging to be the most effective. Vacuum packaging is also more effective than a CO_2 or N_2 atmosphere for the storage of cooked turkey or pork (Nolan *et al* 1989).

In addition to the three types of antioxidant described above, 'peroxide destroyers' react with hydroperoxides to give stable products by non-radical mechanisms (Frankel 1980). Sulphur-compounds, phosphites and phosphines fall into this category, but are not established as safe for use in foodstuffs. Some antioxidants also act by scavenging singlet oxygen, either by quenching or irreversible reactions; these include the tocopherols and carotene (Porter 1980; Houlihan and Ho 1985).

Antioxidants are frequently used in combination; for instance, the inclusion of both Type I and Type II antioxidants generally gives synergistic effects as both initiation and propagation steps are then inhibited (Frankel 1980).

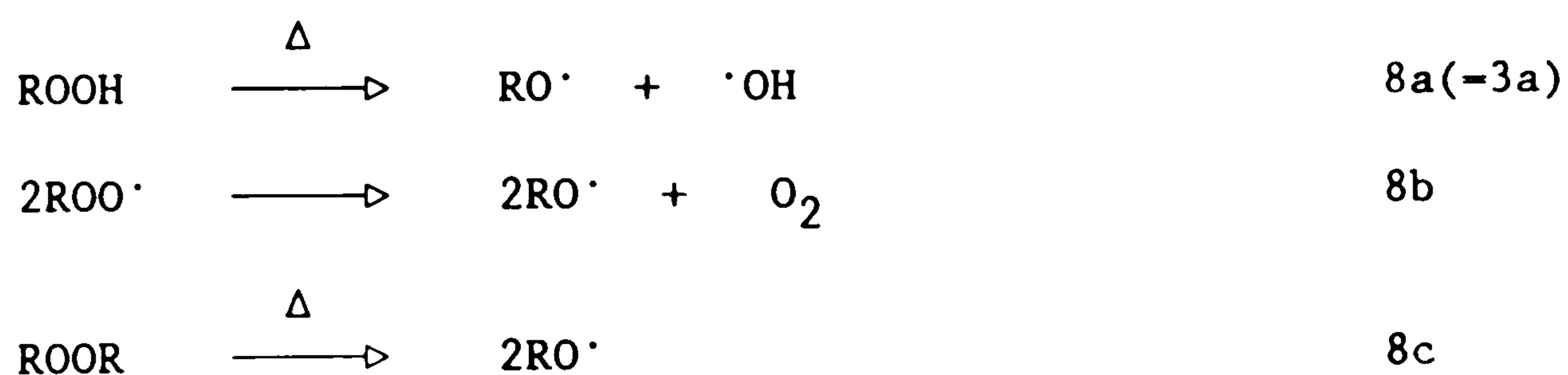
e) **Water content**

The water content of foods has a major effect on their susceptibility to oxidation; foods with very low moisture contents (< 2-3%) are prone to rapid lipid oxidation, while increasing water activity can initially retard oxidation (Labuza 1971; Karel 1980). The oxidation rate drops to a minimum (at a_w = *ca* 0.3) before increasing to a second maximum (a_w = 0.55 to 0.85); a_w = vapour pressure of H_2O in the food compared with that of pure water (Labuza 1971). Several mechanisms may contribute to the

suppression of lipid oxidation as water content increases; water undergoes hydrogen-bonding with hydroperoxides at the water-lipid interface and, thus, interferes with their bimolecular decomposition, and the pro-oxidative effect of any trace metals present is lowered by hydration. These two factors inhibit lipid oxidation by slowing initiation reactions. It is also possible that additional mobility in an aqueous system may increase the chance of collision between free radicals and hence promote termination reactions. It has also been suggested that water may act as an antioxidant by physically excluding oxygen from a food, as the rate of oxidation has been observed to increase markedly when surface water content was reduced below a monomolecular layer (Koch 1962; Labuza 1971). The promotion of oxidation at high water contents may be due to an increase in the mobility and availability of catalysts (Labuza 1971; Karel 1980).

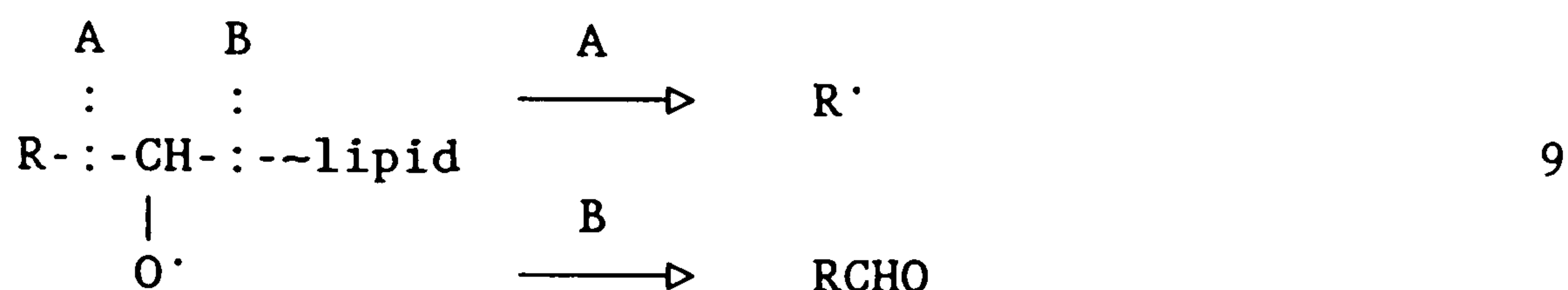
1.2.1.2 Breakdown of hydroperoxides

Lipid hydroperoxides themselves are odourless and tasteless (Henick *et al* 1954). However, they are the precursors of a variety of volatile odour compounds, some of which have characteristic aromas at concentrations of less than 1 part in 10^9. Hydroperoxides are readily decomposed, thermally or in the presence of metal ions, to give alkoxy radicals, from which most of these volatile oxidation products arise. Alkoxy radicals are formed by the branching reactions described in Figure 1.2A, including homolytic cleavage (8a, = 3a in Fig. 1.2A), and also by the interaction of two peroxy radicals (8b), or cleavage of a peroxide (8c), as described by Bell *et al* (1951)



Metal catalysed decomposition of hydroperoxides to give alkoxy radicals occurs by the reactions already described (6a, 6b).

Secondary alkoxy radicals (RCH(O˙)~lipid), formed during the first stages of lipid autoxidation, may cleave or further react to give a variety of species, as reviewed in detail by Frankel (1982) and Grosch (1982). Scission occurs primarily adjacent to the carbon carrying the alkoxy radical, at A or B in reaction 9.



The nature of the volatile products formed from a particular hydroperoxide depends on the composition of R and whether cleavage occurs at A or B. R may be saturated, monounsaturated or polyunsaturated depending on the degree of unsaturation of the fatty acid and the position of the hydroperoxy group in relation to any double bonds (see Figs. 1.2B to E). Some of the pathways by which lipid hydroperoxides can break down to give volatile compounds are shown in Figures 1.2G,H for saturated and monounsaturated fatty acids, and in Figures 1.2I,J for the allylic and diene systems derived from polyunsaturated fatty acids (Grosch 1982).

Where R is saturated, scission at B yields a saturated aldehyde, while cleavage at A gives an alkyl radical. The latter can form an alkane or react with oxygen to give a primary hydroperoxide, which can undergo similar reactions to those of the secondary hydroperoxide of the lipid giving the saturated alcohol or aldehyde (Fig. 1.2G).

Where R contains one double bond, conjugated to the alkoxy radical (such as arises from the 8- and 9-hydroperoxides of oleic acid) the volatile products include monounsaturated compounds. Cleavage at B yields a 2-alkenal while scission at A gives a 1-alkenyl radical; this latter species is extremely labile and further reacts to give the 1-alkene, 1-alkyne or another aldehyde (Frankel 1985).

The more highly unsaturated fatty acids, such as linolenic and arachidonic acids, give allylic hydroperoxides (the 13-hydroperoxide and 9- and 12- hydroperoxides respectively). In this

Figure 1.2G: Breakdown pathways for lipid hydroperoxides when the end of the chain (R) is saturated (Grosch 1982).

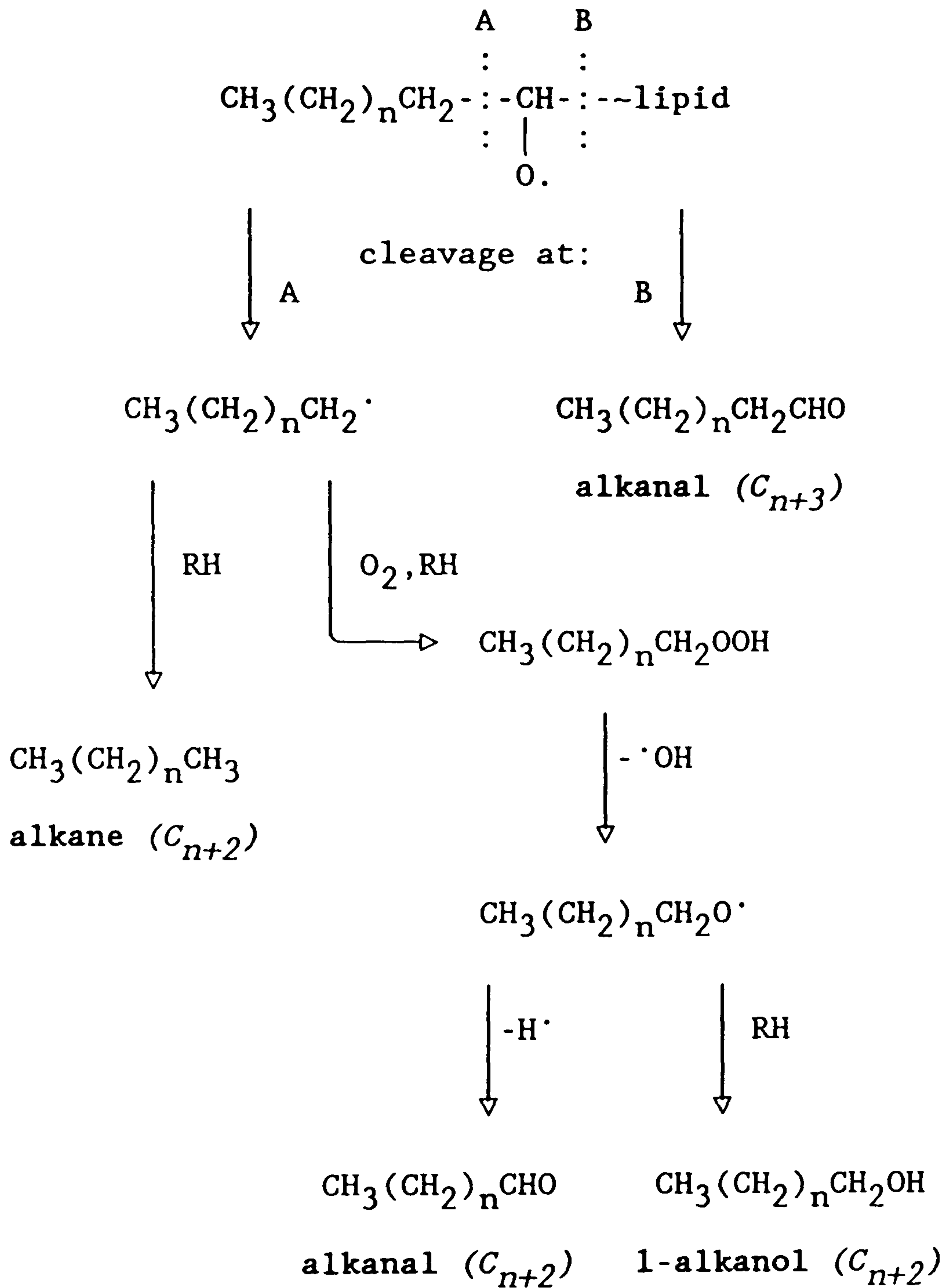


Figure 1.2H: Breakdown pathways for lipid hydroperoxides when R contains an ene system (Grosch 1982).

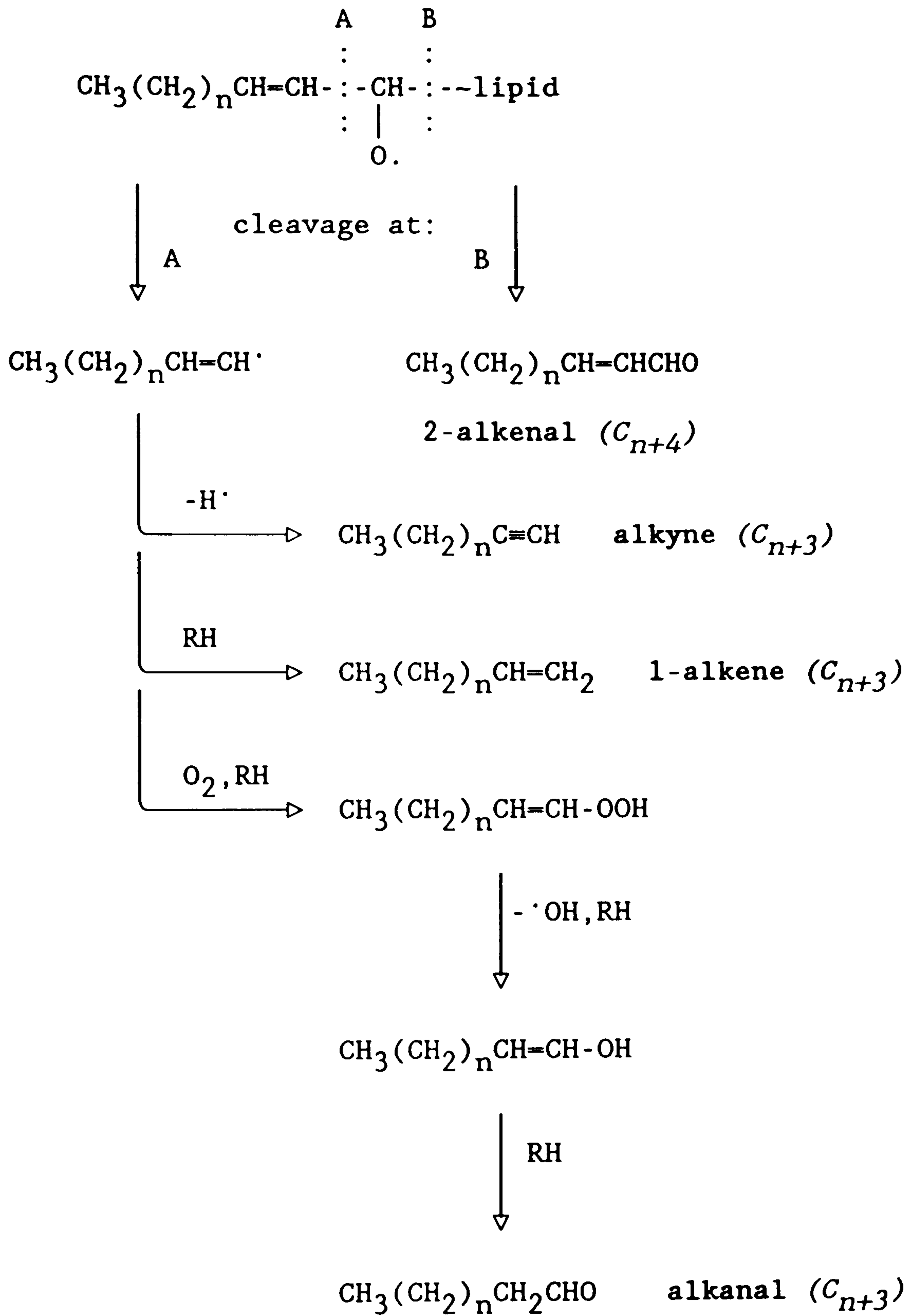


Figure 1.21: Breakdown pathways for lipid hydroperoxides when R contains an allylic system (Grosch 1982).

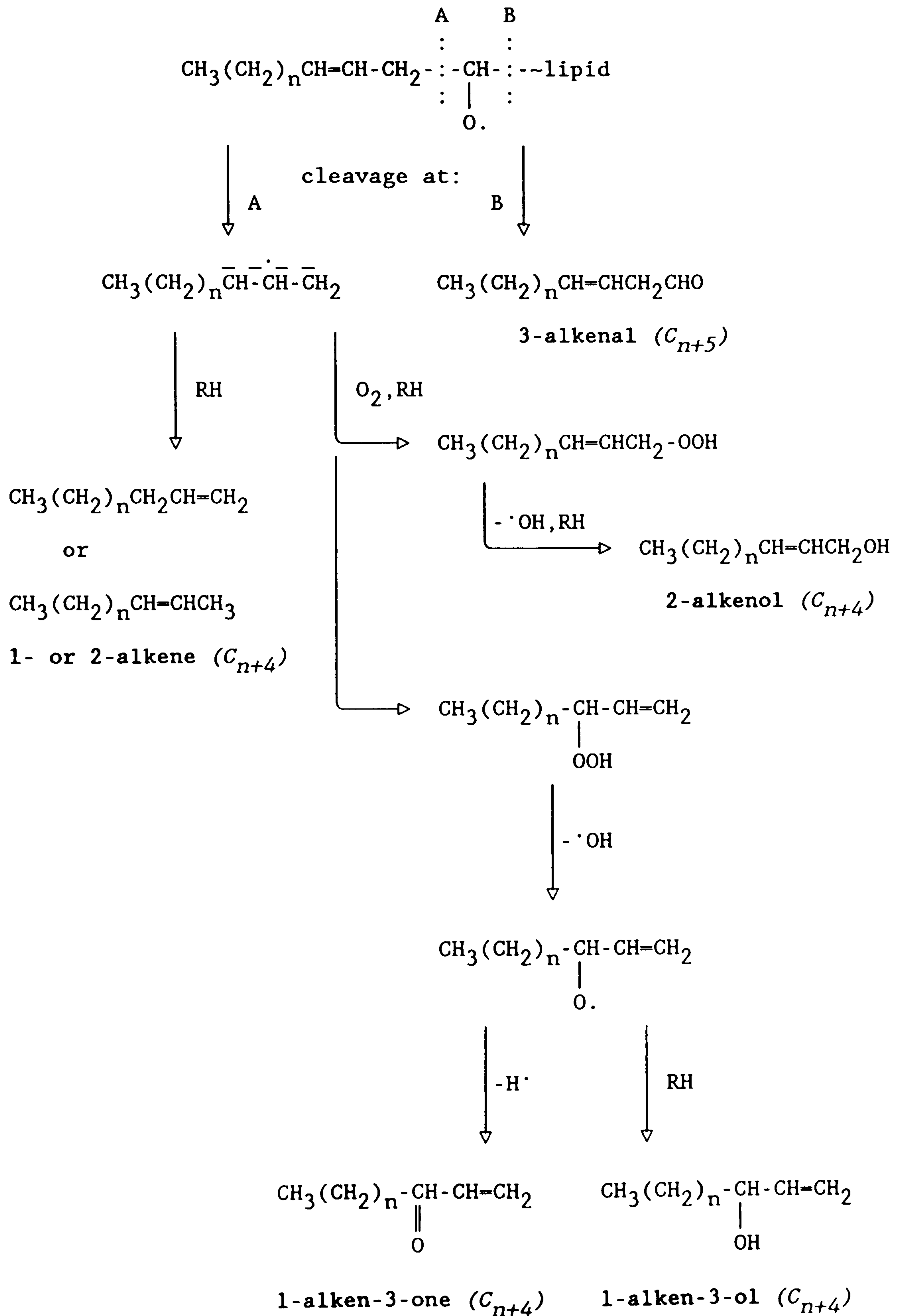
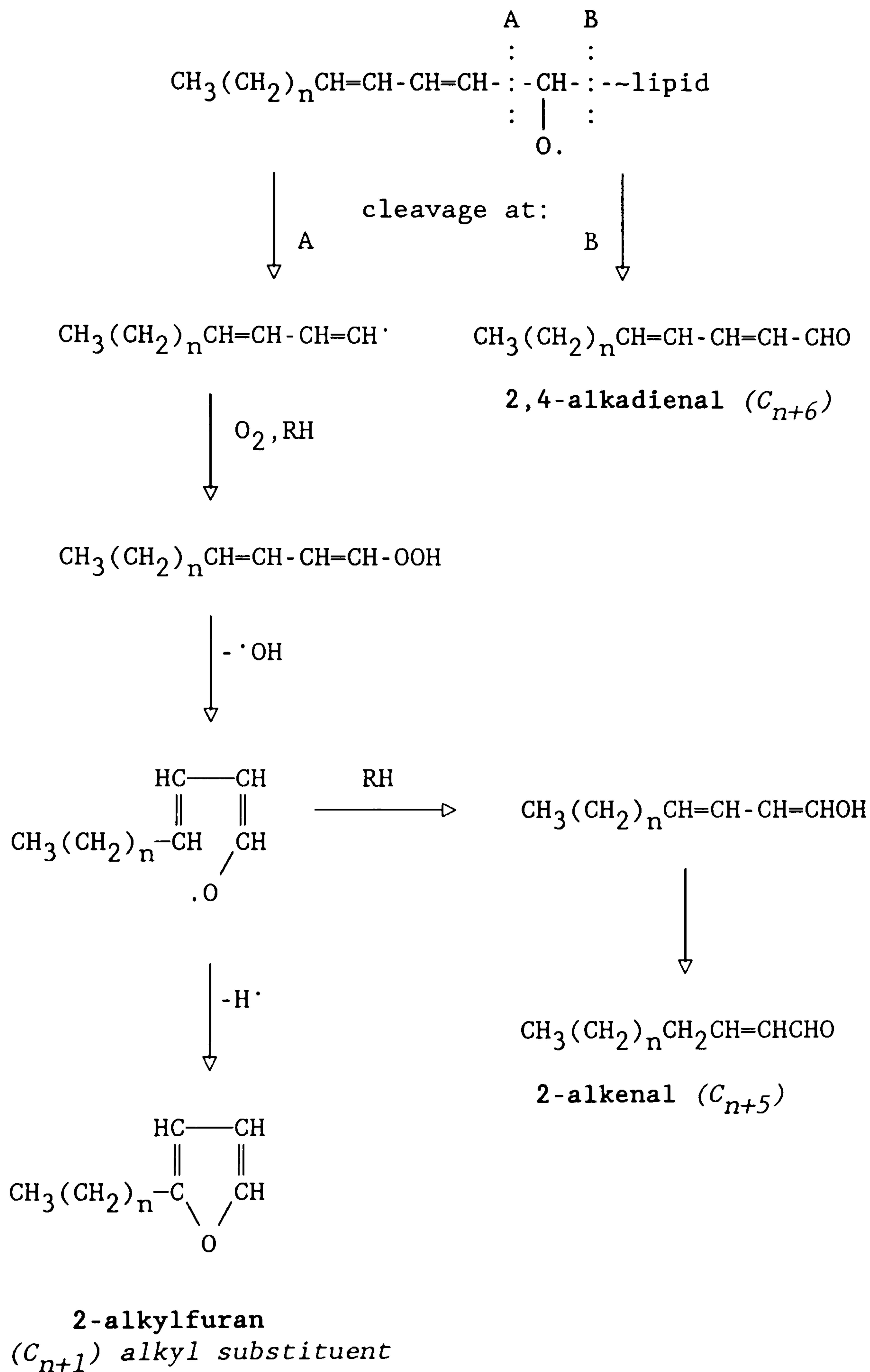


Figure 1.2J: Breakdown pathways for lipid hydroperoxides when R contains an diene system (Grosch 1982).



case scission of the alkoxy radical at B gives the 3-alkenal while cleavage at A yields a delocalized radical capable of giving a range of further products (Fig. 1.2I).

In several of the hydroperoxides from linoleic, linolenic and arachidonic acids R contains a conjugated diene system; such hydroperoxides break down to give complex range of volatile products, including dienals and alkyl furans. 2,4-Alkadienals arise from cleavage at B while alkylfurans are among the possible products of scission at A (Fig. 1.2J).

Thus, the expected compounds from any given unsaturated fatty acid can be inferred from a knowledge of the favoured hydroperoxides and the possible breakdown pathways. Badings (1970) has shown that many, but not all, of the autoxidation products of a fatty acid can be forecast in this way. Most of the predicted oxidation products are detected, but which of these pathways is dominant depends on conditions of temperature, oxygen pressure, the state of the lipid and the presence or absence of catalysts (Grosch 1982). The formation of additional compounds is due to secondary oxidation reactions of the initial products of hydroperoxide breakdown. Some of these reactions are included in Figures 1.2G-J, but as autoxidation progresses these reactions and the range of compounds produced become increasingly complex.

1.2.1.3 Secondary reactions

The autoxidation of fatty acids and esters yields a great many compounds, only a limited number of which can be explained directly by the breakdown of the hydroperoxides. As autoxidation progresses, both the hydroperoxides and the volatile products are subject to further oxidative attack to produce volatile compounds whose mechanisms of formation are often obscure. The extent of secondary oxidation increases with degree of unsaturation and increasing temperature (Nawar 1985). The effect of heat on lipid oxidation will be discussed later in this Chapter (Section 1.2.2.2).

Hydroperoxides undergo secondary oxidation to yield a variety of polyoxygenated products containing keto, epoxy, hydroxy and

hydroperoxy groups (Frankel 1985). In autoxidized methyl linoleate, the major secondary products are hydroxy-cyclic peroxides and dihydroperoxides (Neff *et al* 1981). Hydroxy-cyclic peroxides are formed from the 12- and 13-hydroperoxides, as illustrated in Figure 1.2F (Haverkamp Begemann 1968). The dihydroperoxides are obtained from the 9- and 16-hydroperoxides by hydrogen abstraction and radical formation at the unreacted 1,4-pentadiene system (Neff *et al* 1981). These secondary oxidation products may undergo thermal decomposition to give volatile products containing epoxy, keto groups etc. (Frankel 1985).

The oxidation of several typical volatile products of the autoxidation of linoleate and linolenate have been investigated at temperatures up to 50°C (Lillard and Day 1964; Michalski and Hammond 1972). 2-Nonenal and the 2,4-dienals are most susceptible to oxidation, giving a range of volatile products in addition to the corresponding acids and polymeric material. No additional volatile compounds were derived from the oxidation of n-aldehydes or 1-pentanol, although both decomposed to the corresponding acid. 1-Octen-3-ol oxidized to give 1-octen-3-one, a compound which both groups found to be particularly resistant to oxidation. It was demonstrated that many of the products from the autoxidation of methyl linoleate and methyl linolenate, which were unaccountable by hydroperoxide decomposition, could result from the oxidation of unsaturated aldehydes predicted from primary oxidation processes (Lillard and Day 1964).

Degradation of the products of autoxidation can occur by mechanisms other than oxidation. In the presence of water, 2-alkenals and 2,4-decadienal can undergo hydration and retro-aldol condensation reactions to give a different range of products to those expected from autoxidation (Josephson and Lindsay 1987a). It is suggested that such water-mediated, non-oxidative reactions can account for unusual sites of unsaturation; for example, the presence of *trans,cis*-2,5-undecadienal and *cis*-4-decenal in cooked chicken (Harkes and Begemann 1974) can be attributed to retro-aldol related degradations of *trans,cis,cis*-2,4,7-tridecatrienal and *trans,cis*-2,6-dodecadienal from arachidonic and linoleic acids respectively (Josephson and Lindsay 1987a). *Cis*-4-heptenal in cooked potatoes is thought to arise from *trans,cis*-2,6-nonadienal by a similar mechanism (Josephson and Lindsay 1987b).

1.2.2 THERMAL DEGRADATION OF LIPIDS

The exposure of food lipids to heat treatment results in a variety of chemical changes and yields a wide range of volatile compounds. The reactions occurring are a combination of thermolytic (non-oxidative) breakdown reactions, oxidation reactions and reactions with other components of the food. This Section will discuss the contribution of thermolytic and oxidation reactions to the thermal degradation of lipids in the absence of other components.

1.2.2.1 Thermolytic reactions

The thermolytic degradation of unsaturated fatty acids in the absence of oxygen yields dimeric and cyclic compounds as the dominant products, with hydrocarbons and various esters also formed at high temperatures (Artman and Smith 1972; Nawar 1985). It is thought that free radicals are generated by homolytic cleavage of C-C linkages near the double bond, which recombine to give dimers and cyclic compounds. Cyclic compounds can also arise from unsaturated fatty acids via a Diels-Alder reaction between a double bond and a conjugated diene to give tetra-substituted cyclohexenes (Nawar 1985). When fats are heated in the presence of moisture, hydrolysis occurs yielding free fatty acids; Nawar (1969) concludes that a preference for the hydrolysis of shorter chain and unsaturated fatty acids may be explained by their greater solubility in water.

Saturated lipids are relatively stable to high temperatures under non-oxidative conditions, and temperatures of 200 - 700°C are required to cause decomposition. Only small quantities of breakdown products are derived from triacylglycerols treated at 180°C; these comprise mainly the component fatty acids, diacylglycerols, alkanes and alkenes, symmetric ketones, oxypropyl esters, propenediol and propanediol diesters, acrolein, CO and CO_2. The routes of formation of these compounds are reviewed by Nawar (1985). Sensitive analytical techniques are required to detect these products and the thermolytic degradation of saturated

fatty acids would be expected to contribute little at more moderate temperatures. In practise, such non-oxidative conditions are unlikely to exist in heated food systems.

1.2.2.2 Thermal oxidation

Evidence suggests that the mechanisms of oxidation of unsaturated fatty acid moieties at elevated temperatures are basically the same as those established for autoxidation at ambient temperatures. Analysis of the hydroperoxides from oleate, linoleate and linolenate, at temperatures ranging from 25 to 80°C, indicates that, qualitatively, the same isomers are formed. At higher temperatures the identification of individual hydroperoxides becomes very difficult due to their extreme lability; their formation and destruction is extremely rapid. Nevertheless, hydroperoxides have been shown to be present at temperatures up to 250°C (Nawar 1985).

Effect of heat on mechanisms of autoxidation

Despite the similarities in oxidation pathways the odour qualities of the volatile compounds produced by thermal oxidation may differ markedly from those produced by low temperature oxidation. Heat affects the mechanisms of oxidation in several ways.

a) Reactivity of hydroperoxides

At elevated temperatures, the rapid rate of oxidation ensures that the overall amount of volatile products is much greater than produced at ambient temperatures (Nawar 1989). Under these conditions, the rate of hydroperoxide decomposition exceeds that of formation; thus, in thermally oxidized fat hydroperoxides have a short lifetime and exist only at low concentrations. One consequence of the increased lability of hydroperoxides is that the pro-oxidative effect of metals and other catalysts is much reduced (Grosch 1982).

b) **Competition between pathways**

Where there is a choice of breakdown route, this may be influenced by temperature (Nawar 1985). Swoboda and Lea (1965) have observed that, while hexanal is the major volatile product of low temperature autoxidation of safflower oil, 2,4-decadienal predominates at higher temperatures. Similarly, oxidation of oleate at moderate temperatures yields mainly the saturated aldehydes, while elevated temperatures can produce large proportions of alkenals (Kimoto and Gaddis 1969). These observations may be explained by the suggestion of Kimoto and Gaddis (1969) that the C-C linkage between an alkoxy radical and a double bond is particularly susceptible to cleavage; thus, at low temperatures, this type of cleavage is preferred to scission of an alkoxy – methylene linkage. Cleavage of the four possible alkoxy radicals from oleate between the alkoxy carbon and a double bond would yield saturated aldehydes or unsaturated hydrocarbons depending on the relative positions of the alkoxy group and the double bond (see Fig. 1.2B), while cleavage of the alkoxy – methylene linkage would give unsaturated aldehydes and saturated hydrocarbons as main products (see mechanisms shown in Figures 1.2G and H). It is suggested that, at low temperatures, the route of scission requiring the lower activation energy predominates, but that more vigorous conditions supply sufficient energy for the less preferred route of cleavage to give increased amounts of alkenals.

The amount of any given product formed during oxidation is determined by the net balance between competing reactions; as the rates of these may be affected differently by temperature, the overall pattern of volatile products will also be altered (Nawar 1989).

c) **Secondary oxidation**

At elevated temperatures the primary products of lipid oxidation may be unstable and secondary oxidation pathways become more important and extensive than at ambient temperatures. Thus, although the initial oxidation pathways appear to be the same over a wide range of temperatures, the precise routes of formation of the end products from a heated system are frequently difficult to ascertain. The correlation between the products predicted from

hydroperoxide breakdown and those detected diminishes not only with increasing temperature, but also with increasing degree of unsaturation (Nawar 1985).

d) **Isomerisation**

The *cis/trans* isomerisation of oxidation products can also be affected by heat; the diene hydroperoxides formed from linoleic acid are a mixture of *cis,trans* and *trans,trans* isomers, with more of the latter formed at higher temperatures (Gunstone 1984).

e) **Oxidation of saturated fatty acids**

At ambient temperatures lipid oxidation occurs largely at the unsaturated fatty acid moieties and saturated fatty acids are largely resistant to oxidation. However, at elevated temperatures (>150°C), oxidation of saturated lipids occurs to give the parent fatty acid and also 2-alkanones, n-alkanals, lactones, n-alkanes and 1-alkenes (Nawar 1985). Like that of the unsaturated fatty acids, oxidation of saturated fatty acids occurs via the formation of monohydroperoxides. While unsaturated fatty acids usually yield a limited number of predictable hydroperoxides, saturated fatty acids may, in theory, undergo oxidative attack at any of the methylene groups. Selke *et al* (1975) showed that each class of compounds, obtained from heating tristearin to 192°C in air, was represented by a homologous series; they suggested that oxidative attack could occur at any of the fatty acid methylene groups. The prevalence of the C_7 - C_{10} homologues was thought to indicate some preference for oxidative attack towards the centre of the chain. Similar conclusions were drawn by Brodnitz (1968) in studies on methyl palmitate.

In contrast, a number of studies (Crossley *et al* 1962; Endres *et al* 1962; Jewell and Nawar 1980) have reported a prevalence of oxidative products with chain lengths near that of the parent fatty acid; the 2-alkanones largely comprised the C_{n-1}, C_{n-2} and C_{n-3} homologues in decreasing abundance. This was thought to suggest that oxidation occurs mainly at positions α, β or γ to the acid (or ester group) to give the three corresponding hydroperoxides. Breakdown pathways of these hydroperoxides are postulated which explain the formation of the detected products.

The products formed in any heated fat will depend on the conditions used, especially temperature, and it has been suggested by Nawar (1985) that the above contrasting results may either be due to the use of different oxidative conditions and techniques, or to the rapid decomposition of the higher hydroperoxides at the elevated temperatures used giving the observed pattern of homologues.

The pattern of volatile compounds from tristearin can be clearly discerned amongst the volatiles detected by GC from a heated triolein, tristearin mixture (Selke *et al* 1977); thus, the oxidation products of saturated fatty acids might be expected to contribute to the volatile compounds produced on heating natural oils and fats.

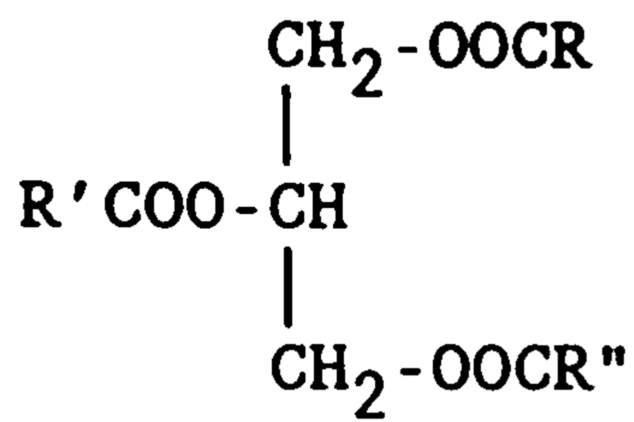
1.2.3 DEGRADATION REACTIONS OF PHOSPHOLIPIDS

Phospholipids possess a number of distinct structural and functional properties, quite different from those of the more abundant triglycerides. These dissimilarities affect the contribution made by phospholipids to the flavour and aroma of foods. The main features characteristic of phospholipids may be listed as follows:

1. While triglycerides comprise a glycerol molecule to which is esterified three fatty acids, most phospholipids contain two fatty acids and a polar group, made up of a phosphate and a polar organic group. The two most abundant phospholipids contain choline and ethanolamine as the polar moieties. The presence of this hydrophilic group causes phospholipids to be more miscible with water than triglycerides. Figure 1.2K compares the structures of triglyceride and the more common phospholipids.

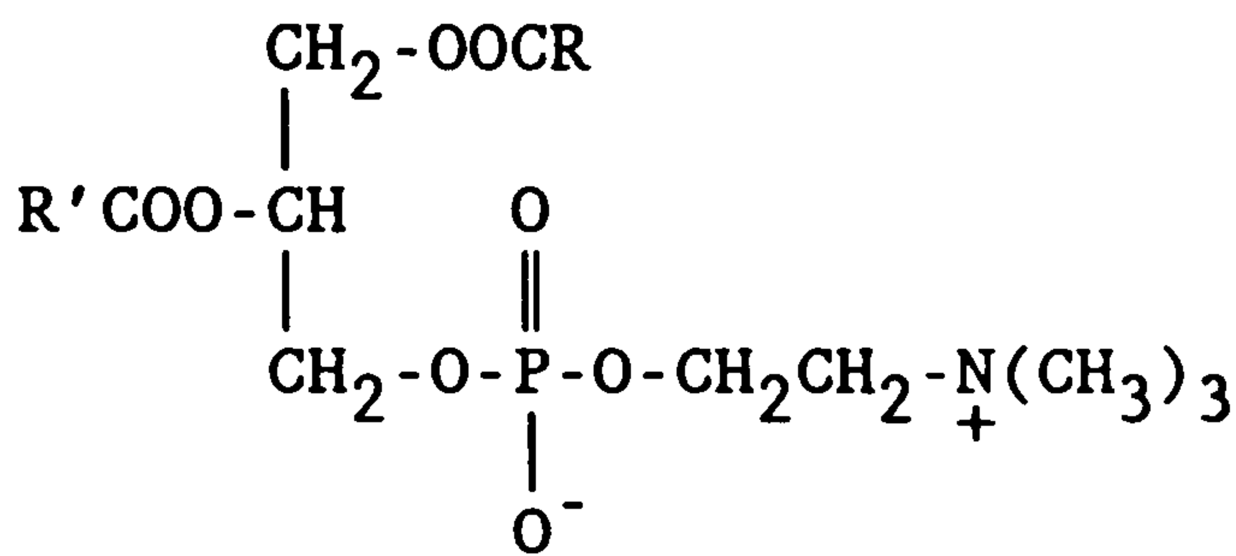
2. Phospholipids usually contain a higher proportion of polyunsaturated fatty acids than do triglycerides, with further differences frequently occurring between individual

Figure 1.2K: Structure of triglyceride and the principal phospholipids (Christie 1973) together with their % abundance [] in bovine *Longissimus dorsi* (Christie 1978)

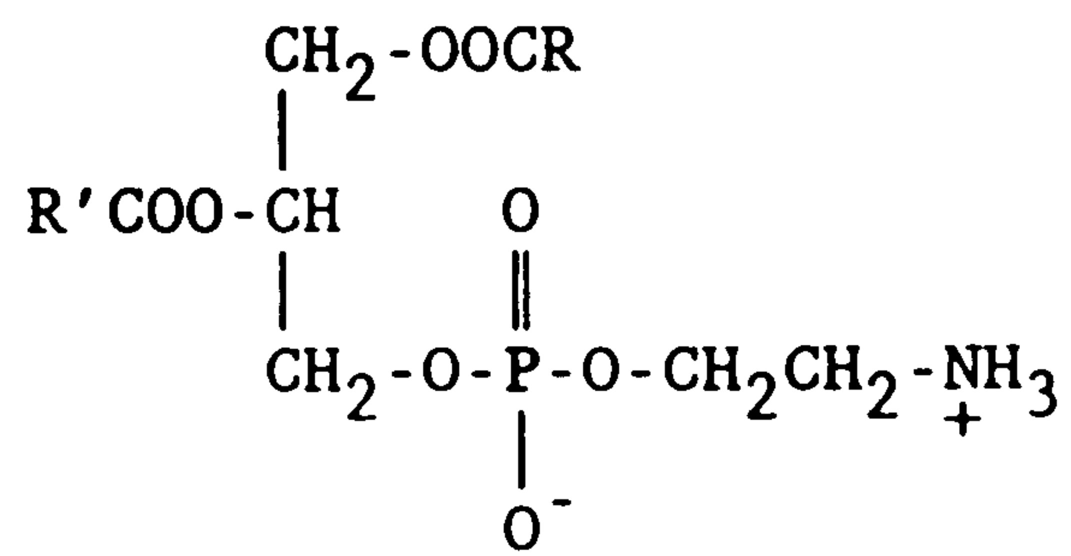


triacylglycerol
(triglyceride)
[2-15% wet tissue]

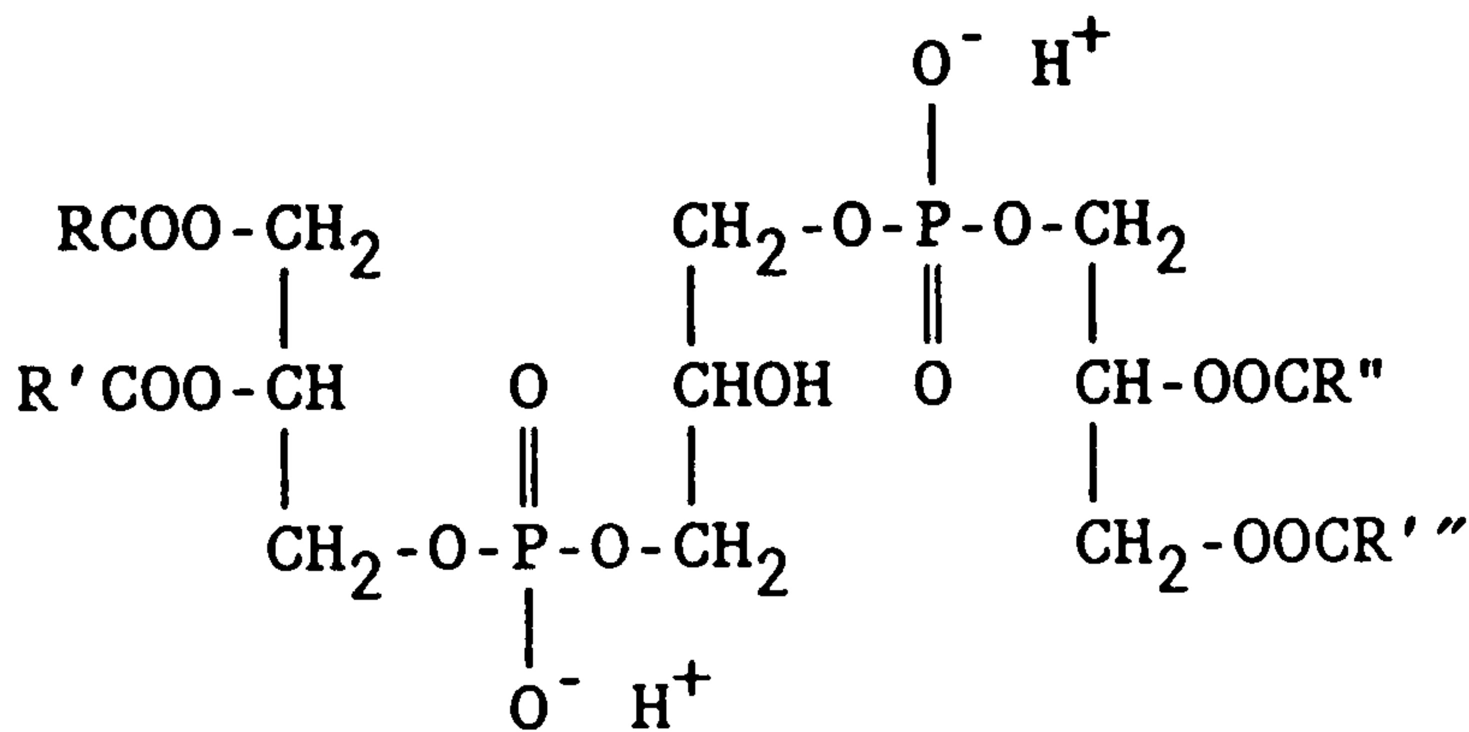
Phospholipids [0.4-1.0% wet tissue]



phosphatidylcholine
(PC, lecithin)
[46.5% lipid phosphorus (P)]

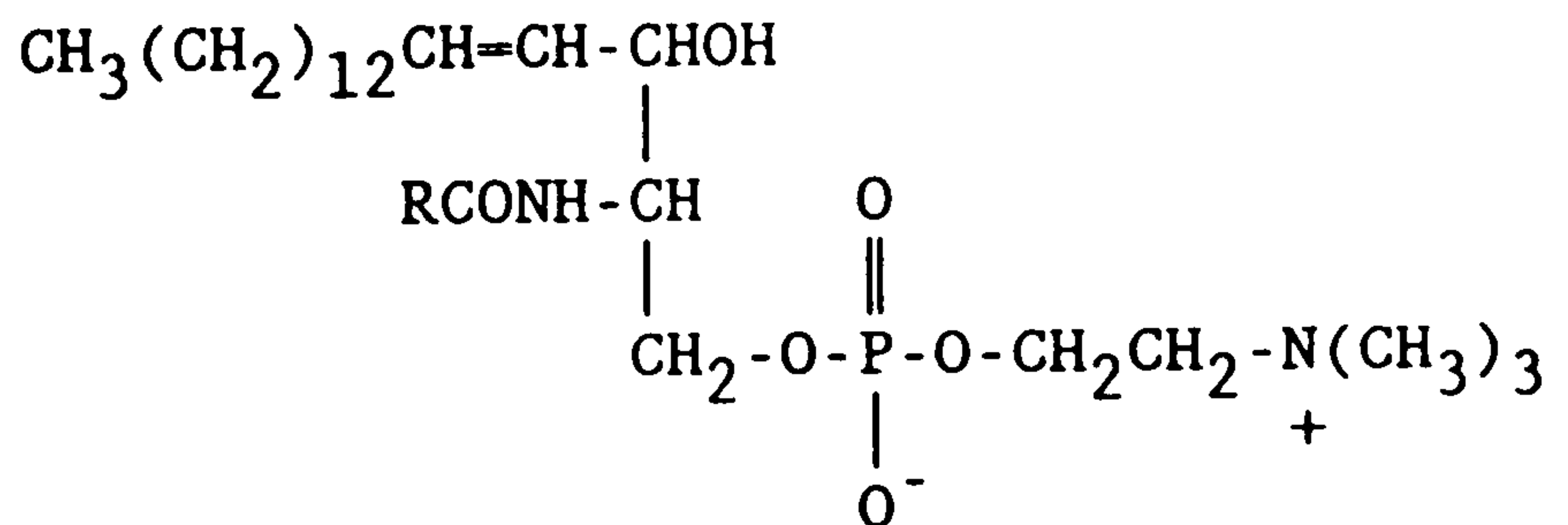


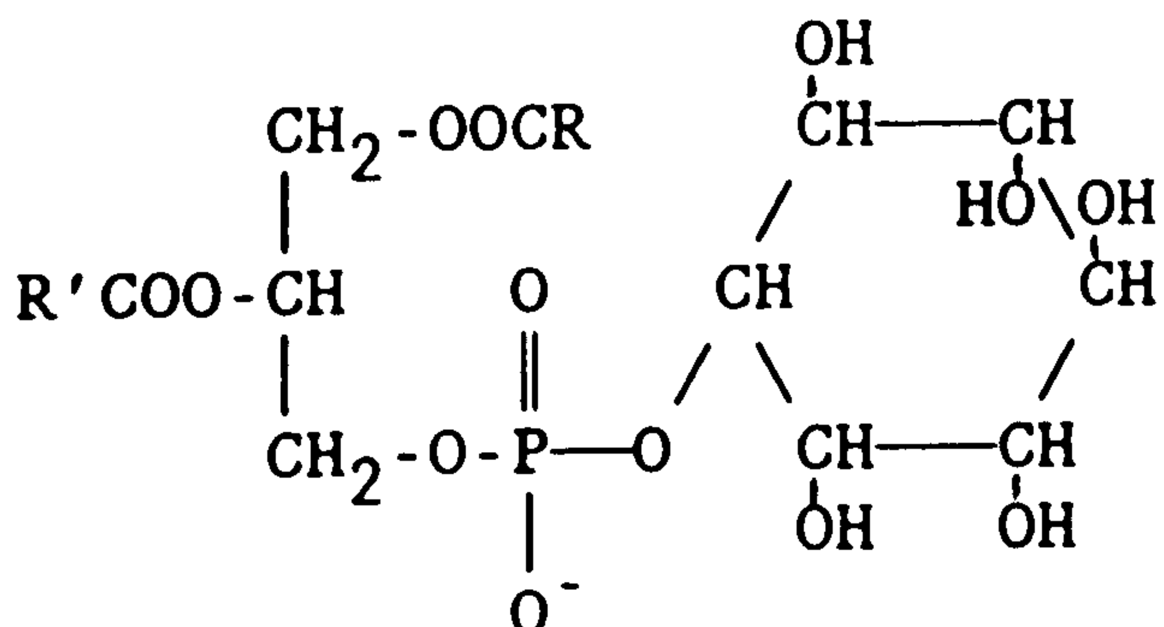
phosphatidylethanolamine
(PE, cephalin)
[26.6% lipid P]



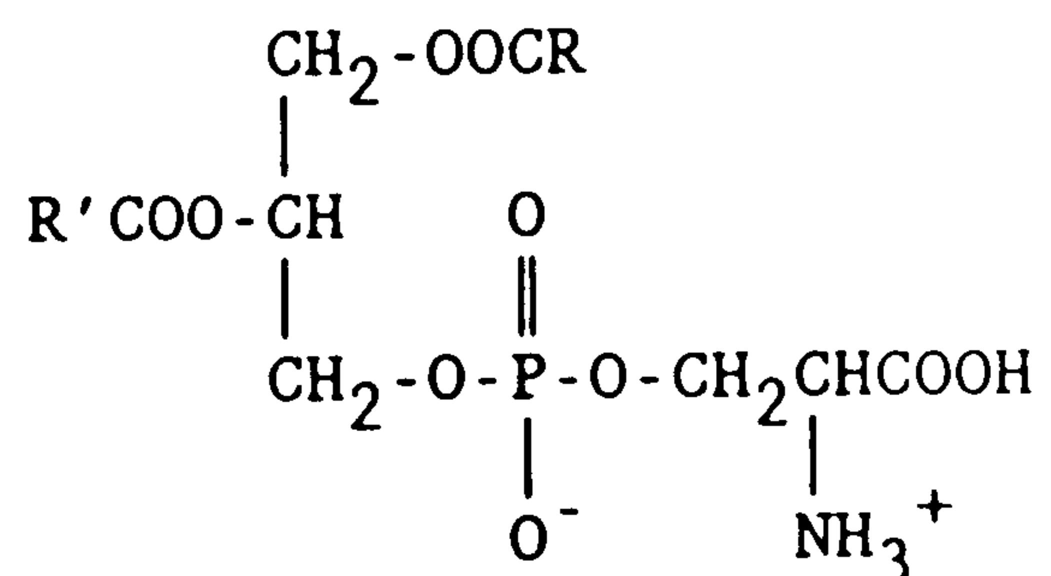
cardiolipin
[8.9% lipid P]

sphingomyelin
[4.5% lipid P]





phosphatidylinositol
[5.6% lipid P]



phosphatidylserine
[4.1% lipid P]

Table 1.2d : Fatty acid composition of triglyceride (TG) and phospholipids in beef and chicken muscles and in egg lipid (Hornstein *et al* 1961; Marion *et al* 1967; Privett *et al* 1962).

Fatty acid[a]	Fatty acid composition (% fatty acids)								
	Beef			Chicken			Egg		
	TG	PC[b]	PE	TG	PC	PE	TG	PC	PE
10:0	0.1	-	-	-					
12:0	0.1	-	-	-		0.4			
14:0	2.2	3.6	1.4	0.7	0.1	0.3			
14:1	1.0	1.1	0.6						
14:2	0.6	1.3	0.6		4.2	6.2			
16:0	27.5	21.0	2.8	25.7	34.3	6.7	22.5	37.0	21.6
16:1	4.7	2.4	1.8	6.8			7.3	0.6	tr
16:2						2.6			
18:0	16.9	6.6	27.4	7.4	9.1	28.6	7.5	12.4	32.5
18:1	41.3	26.9	13.8	38.5	24.7	17.1	44.7	31.4	17.3
18:2	4.4	23.6	16.2	19.4	15.5	8.9	15.4	12.0	7.0
18:3	1.1	1.7	2.1	0.5			1.3	1.0	2.0
20:3	-	3.2	-						
20:4	0.1	8.6	33.3	1.1	7.8	21.4	0.5	2.7	10.2
20:5				-	0.2	0.3	c	c	c
24:1				-	2.5	3.9			
22:4					0.2	0.6			
22:5				-	0.1	0.4	0.2[c]	0.8[c]	3.0[c]
22:6				-	0.1	0.5	0.6	2.1	6.4
Total	100	100	100	100.1	98.8	97.9	100	100	100
Σ sat	46.8	31.2	31.6	33.8	43.5	36.0	30.0	49.4	54.1
Σ mono	47.0	30.4	16.2	45.3	27.2	21.0	52.0	32.0	17.3
Σ 2db[d]	5.0	24.9	16.8	19.4	19.7	17.7	15.4	12.0	7.0
Σ ≥3db	1.2	13.5	35.4	1.6	8.4	23.2	2.6	6.6	21.6

a Some fatty acids were only tentatively identified.
b Beef PC fraction includes sphingomyelins.
c Fatty acids 20:5 and 22:5 were quantified together.
d db = double bonds.

phospholipid classes. Table 1.2d shows that phosphatidylcholine (PC) and phosphatidylethanolamine (PE), the two most abundant phospholipids, contain a higher proportion of the highly unsaturated fatty acids than triglyceride, whether they originate from beef or chicken muscle or egg lipids. Of these two phospholipids, PE is especially high in fatty acids containing three or more double bonds.

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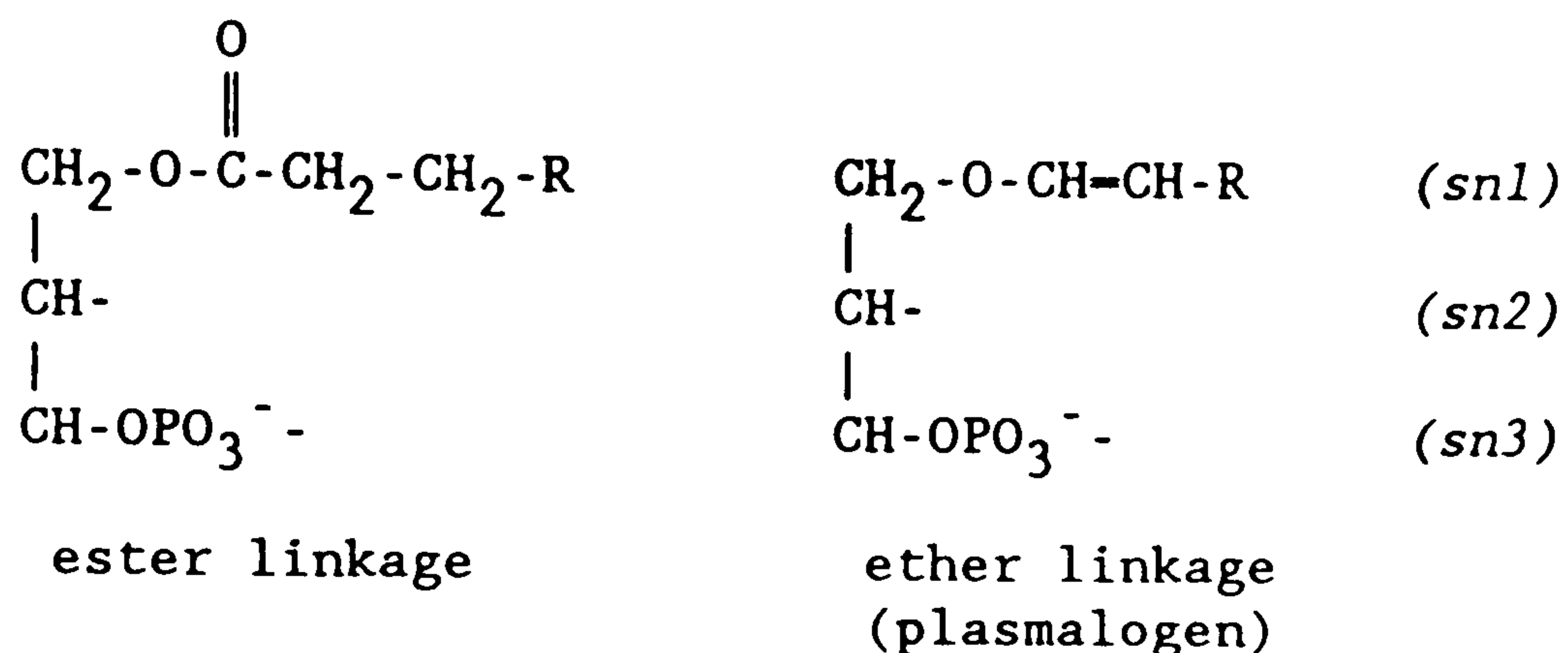
3. While most phospholipid fatty acids are attached to the glycerol backbone by the ester linkage typical of triglycerides, some fatty acids in the *sn1* position are attached by an ether linkage; this gives the plasmalogen form of the phospholipid (Christie 1973).



4. In animal tissues, most triglycerides are deposited in adipose tissue for the storage of energy or are present as inter- or intramuscular lipids (marbling fat). In contrast, phospholipids exist mainly in the cell walls and are key components of the membrane structure. As such, they are in intimate contact with the aqueous contents of the cells.

The oxidation of phospholipids, whether by autoxidation or thermal oxidation, proceeds by the same free radical reactions as those described for lipids in general. However, the high concentration of polyunsaturated fatty acids in phospholipids makes them particularly susceptible to oxidative attack and this ensures that their oxidation is of considerable importance in the generation of off-flavours in foods. In addition, the presence of characteristic polar moieties causes other reactions characteristic of the phospholipid concerned.

It was observed by Lea, in 1957, that the presence of highly unsaturated fatty acids in phospholipids not only increases their susceptibility to oxidation but also leads to the development of strongly flavoured decomposition products early in the oxidation process. Indeed, as little as 0.1% of phospholipid in soybean oil can produce characteristic off-flavours upon oxidation. The removal of phospholipids is known to be a prerequisite for the production of soybean oil with good colour and flavour

stabilities, as traces of phospholipid will readily oxidize to give a brown colour and "fishy" off-flavours (Smouse 1985).

Hornstein *et al* (1961) separated the lipids from raw beef and pork into triglycerides and two phospholipid fractions and exposed them to air for seven days; rancid odours developed more rapidly in the phospholipid fractions than in the triglyceride. These results agree with those of El-Gharbawi and Dugan (1965) who studied the oxidation of the lipids of freeze-dried raw beef during storage. Oxidation occurs in two stages (or at two rates); the phospholipids oxidize first and the more saturated neutral fat later. The considerable loss of polyunsaturated fatty acids from the oxidized phospholipids is in accord with the faster rate of oxidation of these fatty acids.

Phospholipids have been particularly implicated in the generation of rancidity in stored cooked meats; the rancid off-flavour formed under these circumstances has become known as 'warmed over flavour'. It was observed by Tims and Watts (1958) that the rate of oxidation of cooked meat stored at 5-7 ^{o}C greatly exceeded that of the raw meat for beef, veal, lamb and chicken. As similar oxidation had not been observed in the fat extracted from cooked meat, it was suggested that the rancidity was caused by tissue-bound phospholipids. Similar conclusions were drawn by Wilson *et al* (1976) who found that 'red' muscles suffered more oxidation than 'white' muscles from various cooked meats stored at 4°C, and reported that the TBA values and phospholipid content were correlated. They suggested that phospholipids played a major role in the development of 'warmed over flavour' in all the cooked meats investigated except pork.

The cooking of beef has been shown to coincide with oxidation of the constituent lipids, as measured by the TBA test and carbonyl formation (Keller and Kinsella 1973). These effects were concomitant with a loss of phospholipids in some samples. A considerable loss of arachidonic acid (20:4) from the phospholipids is consistent with thermally induced oxidative breakdown of these phospholipids; of the fatty acids detected, arachidonic would be the most labile to oxidation.

The primary reason for the increased susceptibility of phospholipids to oxidation compared with triglycerides appears to

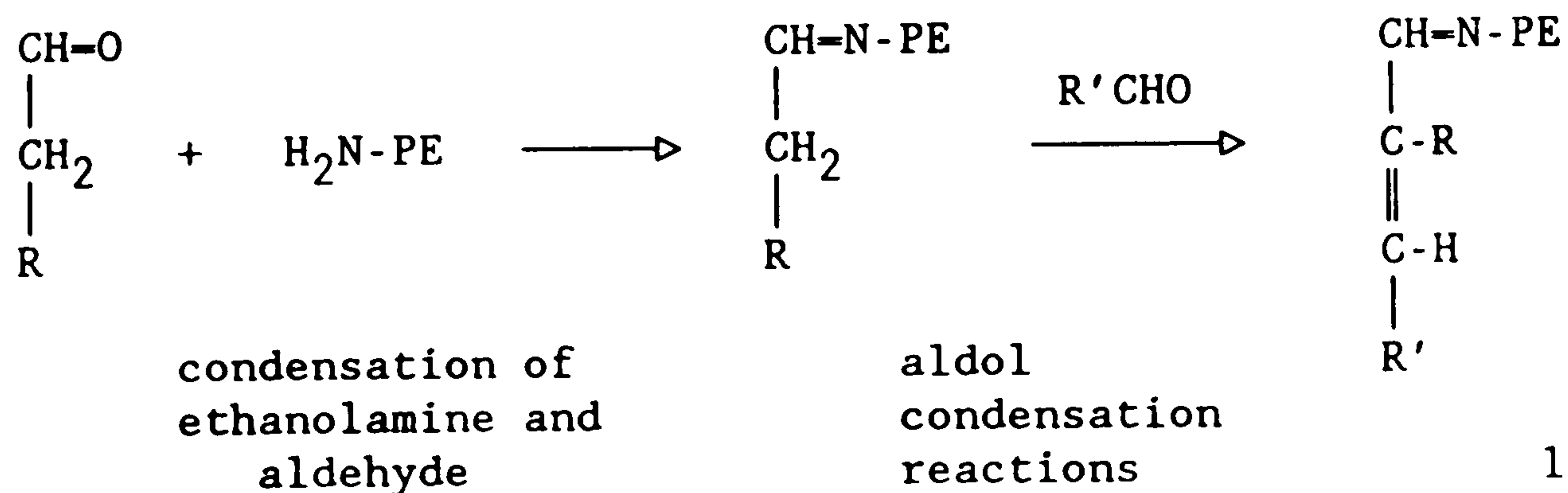
be the high degree of unsaturation of the component fatty acids (see Table 1.2d). However, there are other factors which may also contribute to the ready oxidation of phospholipids. The fact that phospholipids are major components of cell walls may give close contact with catalytic metals and haem compounds (El-Gharbawi and Dugan 1965). Phospholipids also exist in proximity to many cellular enzymes and Lea (1957) describes how a number of enzymes, eg phospholipases, commence the hydrolysis of phospholipids in meat and vegetables immediately the normal life processes of the organism are disrupted. It is probable that the free fatty acids released would be vulnerable to oxidative attack by other enzymes such as lipoxygenases. Some of these enzymes are very resistant to extremes of temperature, whether freezing (Keller and Kinsella 1973) or boiling (Lea 1957), and thus may retain activity during frozen storage and at least during the early stages of cooking.

It has been observed (Lea 1957) that phosphatidylethanolamine (PE, also known as cephalin) oxidizes many times faster than phosphatidylcholine (PC, lecithin). One explanation for this difference is the higher proportion of polyunsaturated fatty acids in PE than PC (Table 1.2d). Early work demonstrated that even when fractions of PC and PE with similar degrees of unsaturation were compared, the PE still absorbed oxygen more rapidly than the PC (Lea 1957). Thus, it was suspected that the polar group of the phospholipid may affect the rate of oxidation. An alternative explanation is that, despite the same overall degree of unsaturation, the PE may have contained a different range of fatty acids, including a higher proportion of polyunsaturated fatty acids.

Heating PE or PC (from egg or soybean) with tripalmitin resulted in the development of a brown colour, with PE producing more colour than PC (Husain *et al* 1986). Colour formation was found to increase with degree of unsaturation when individual fatty acids were heated with either choline or ethanolamine. However, in this case, choline conferred more browning than ethanolamine. Further investigation demonstrated differences in the way in which these two phospholipid polar groups react with the products of lipid oxidation. While choline gave more browning than ethanolamine when heated with methyl linoleate, ethanolamine showed greatly increased reactivity with the conjugated carbonyl derivative. This

observation is consistent with reaction of the carbonyl group with the free amino group of ethanolamine to give brown-coloured products, as previously described by Suyama and Adachi (1979); these products were found to possess ester linkages and presumably retained the glycerol backbone. The products from the browning of PE had lost the amino function, presumably by Maillard type reactions with carbonyl compounds (Husain *et al* 1986). Similar conclusions were drawn by Jewell and Nawar (1980) who compared the volatile products from heated tripalmitin and 1,2-dipalmitoyl-*sn*-glycerol-3-phosphoethanolamine and found a complete absence of aldehydes in the latter; it was suggested that these compounds were undergoing immediate reaction with the ethanolamine group.

Pokorny and co-workers have studied the browning of PE in some detail and divided this reaction into three stages. The first stage involved autoxidation of the constituent fatty acids to give hydroperoxides; the high content of polyunsaturated fatty acids were particularly susceptible to oxidation and were the only fatty acids oxidized at 40 °C. The second stage of the reaction comprised condensation reactions of the ethanolamine amino group with either the hydroperoxides themselves (Pokorny *et al* 1973) or with aldehydes derived from them (reaction 10; Tai *et al* 1974). This reaction was found to be very slow at room temperature but became relatively rapid at temperatures greater than 60 °C, possibly due to the instability of polyunsaturated fatty acid hydroperoxides at elevated temperatures (Tai *et al* 1974). The unstable products of such reactions (Schiff bases) were thought to react further to give brown pigments by polycondensation reactions with aldehyde molecules. The final products did not contain free amino groups, presumably due to their reaction with lipid degradation products (Pokorny 1973).



During the autoxidation of PE the loss of polyunsaturated fatty acids and amino groups is concomitant with an increase in imine derivatives; the brown colour appears to be associated with these N-containing products (Pokorny 1981). These results are consistent with the reaction of the ethanolamine group with carbonyl compounds produced by autoxidation.

In contrast, the substances responsible for the brown colour in heated PC are thought to be 9-oxo-10,12-dienoic and 13-oxo-9,11-dienoic acids (Tomioka and Kaneda 1974a). It was suggested that such carbonyl compounds may be polymerized by catalytic action of the phosphorylcholine group (Tomioka and Kaneda 1974a,b). The resulting brown products retain much of the structure of the original PC and appear to be the products of base-catalyzed aldol condensation reactions (Tomioka and Kaneda 1974b, 1976). Thus, evidence suggests that the browning reactions of PC and PE proceed by different mechanisms.

Although phospholipids have been shown to be extremely susceptible to oxidation, under certain circumstances they have been reported to exhibit an antioxidative effect. Lipids extracted from certain leaves (eg. ryegrass) exhibit unusual oxidative stability (Hudson and Mahgoub 1981). During studies on factors contributing to this phenomenon, these authors compared the effect of PC, PE and two natural plant antioxidants on the rate of oxidation of an unstable fat (lard), as measured by induction period. While PC and PE on their own show the expected pro-oxidative effect, both phospholipids enhance the antioxidative effect of α-tocopherol and quercetin. It is suggested that the presence of a basic nitrogen enables these phospholipids to chelate metal pro-oxidants. This capability would complement the 'free radical chain stopper' activity of α-tocopherol and quercetin.

The additional stability of crude vegetable oils compared with their refined counterparts has also been attributed to the presence of phospholipids in a number of studies reviewed by Houlihan and Ho (1985); phospholipids appear to have little primary antioxidant activity but may act synergistically as a result of chelation of metal catalysts by their nitrogen-containing function. Metal complex formation has also been cited as the cause of the antioxidative effect shown by fractions separated from heated PC and PE; this effect is exhibited by those

fractions insoluble in a polar organic TLC solvent, but not by the soluble fractions and may depend on chelate formation of polymerized products with metals (Husain *et al* 1986).

1.2.4 VOLATILE COMPOUNDS FROM LIPID OXIDATION

The mechanisms of lipid oxidation are responsible for the formation of many of the volatile compounds detected in foods. Some of these substances are key odour impact compounds bestowing either desirable or rancid flavours. Whether or not a compound contributes to the overall aroma or flavour of a food depends upon both its concentration and the minimum level at which it can be detected (aroma or flavour threshold). The thresholds of lipid oxidation products differ widely, even within a homologous series. However, certain compound classes tend to have very much lower thresholds than others. Some of the lowest thresholds are those of vinyl ketones (ie. 1-alken-3-ones), *trans,cis*-2,4-alkadienals and certain non-conjugated *cis*-alkenals (eg. 3-alkenals); saturated aldehydes, 2-alkenals, 1-alkenes and vinyl alcohols possess intermediate thresholds, while those of acids, saturated alcohols and, especially, alkanes are so high that these substances are presumed to have little effect on flavour (Frankel 1985). Typical odour thresholds for the main volatile products of lipid oxidation are summarized in Table 1.2e (Mottram 1987).

Table 1.2e : Approximate odour thresholds (parts in 10^6) of classes of volatile compounds obtained from lipid oxidation (Mottram 1987)

Compound class	Threshold (parts in 10^6)
Alkanes	$> 2 \times 10^3$
Alkenes	1 - 20
Alkylfurans	1 - 10
Alkanols	0.5 - 20
1-Alken-3-ols	0.01 - 4
Alkanals	0.04 - 0.4
trans-2-Alkenals	0.04 - 0.6
Alkadienals	0.001 - 0.1
1-Alken-3-ones	0.0001 - 0.01

In natural lipids, containing a variety of fatty acids, any one oxidation product may be formed from more than one fatty acid and by several of the hydroperoxide breakdown routes described in Sec. 1.2.1.2, Figs. 1.2G-J. Secondary oxidation of hydroperoxides and their degradation products may provide alternative routes of formation, especially where the precursor is highly unsaturated or the reaction takes place at elevated temperatures (see Sec. 1.2.2). Thus, the main pathway of formation for a given compound is often difficult to ascertain. Nevertheless, it seems worthwhile to examine very briefly each of the major classes of compounds derived from lipid oxidation and to consider their possible origin and odour significance.

1.2.4.1 Aldehydes

Many aldehydes, both saturated and unsaturated, are formed during the oxidation of lipids and these compounds contribute to the overall flavour of numerous foodstuffs. In some cases, they provide the characteristic aroma of a food; for instance, *trans,cis*-2,6-nonadienal in cucumbers and *cis*-3-hexenal in tomatoes (Grosch 1982). Many of the rancid off-flavours formed on storage of oils and fats have also been attributed to aldehydes (Forss 1972).

n-Aldehydes, 2-alkenals and 2,4-alkadienals are products of the degradation of hydroperoxides (Sec. 1.2.1.2) and also of secondary free radical oxidation reactions. Other isomers may arise by secondary reactions involving retro-aldol condensation mechanisms (Sec. 1.2.1.3). The more unsaturated aldehydes, eg. 2,4-alkadienals, are necessarily derived from fatty acids with at least two double bonds while linolenic acid can give alkatrienals (Badings 1970). The high reactivity of such aldehydes means that they are especially vulnerable to further degradation reactions.

The odour thresholds of aldehydes in water range from ca. 1 part in 10^6 (for *cis*-2-pentenal) to 1 part in 10^9 (for *trans,cis*-2,6-nonadienal; Grosch 1982). Unsaturated aldehydes with an $\omega 3$ double bond have particularly low odour thresholds. Thus, 3-alkenals, with an 'isolated' double bond, have lower odour thresholds than the corresponding 2-alkenals. This explains the observation that

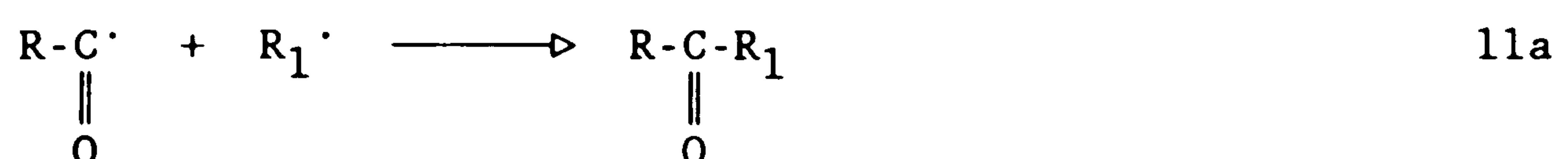
higher peroxide values are required before rancidity develops in linoleate-containing than linolenate-containing fats (Frankel 1985).

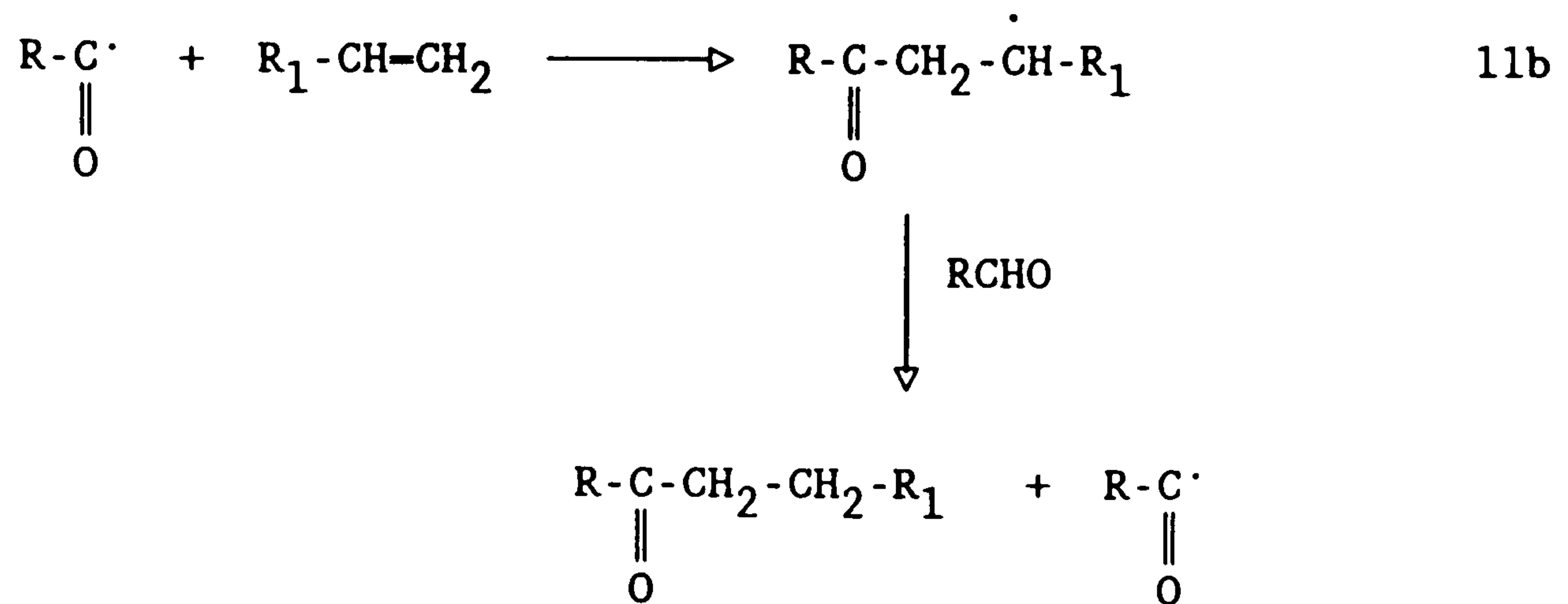
The odours of saturated aldehydes have been described as "sharp" and "green" for pentanal and hexanal, tending to alter to "fatty" and "tallowy" with increasing chain length. Many of the 2-alkenals also possess "fatty" notes, together with "metallic", "walnuts" and "cucumber" odours. The 2,4-alkadienals have "frying" and "oily" odours (Badings 1970).

1.2.4.2 Ketones

Saturated ketones are partly responsible for the distinctive flavour of blue cheese and for off-flavours in Cheddar cheese and various oils, while unsaturated ketones are potent odour compounds and cause characteristic odours in many foodstuffs; 1-octen-3-one can cause a "metallic" off-flavour in butter and oils (Forss 1972) and at lower concentrations is responsible for the flavour of mushrooms (Cronin and Ward 1971). Other alkenones cause the characteristic odours of soybeans and oxidized buttermilk.

Although the formation of 1-alken-3-ones can be predicted by the hydroperoxide breakdown pathways of allylic systems (Sec. 1.2.1.2, Fig. 1.2J) fewer classes of ketones are formed by such routes than aldehydes. However, the thermal oxidation of saturated fatty acids is thought to be a probable route of formation of these compounds (Mottram 1990). Lipid hydroperoxides can give rise to ketoesters which then yield methylketones (2-alkanones) with one less carbon atom than the parent fatty acid (Forss 1972). Another suggested route of formation is via the secondary autoxidation of aldehydes to give acyl radicals; these may react with alkyl radicals or 1-alkenes to give ketones (Mookherjee *et al* 1965).





A mechanism for the formation of 1-octen-3-one has been suggested which is specific to arachidonic acid and therefore to the membrane phospholipids (see Fig. 1.2L; Wilkinson and Stark 1967)

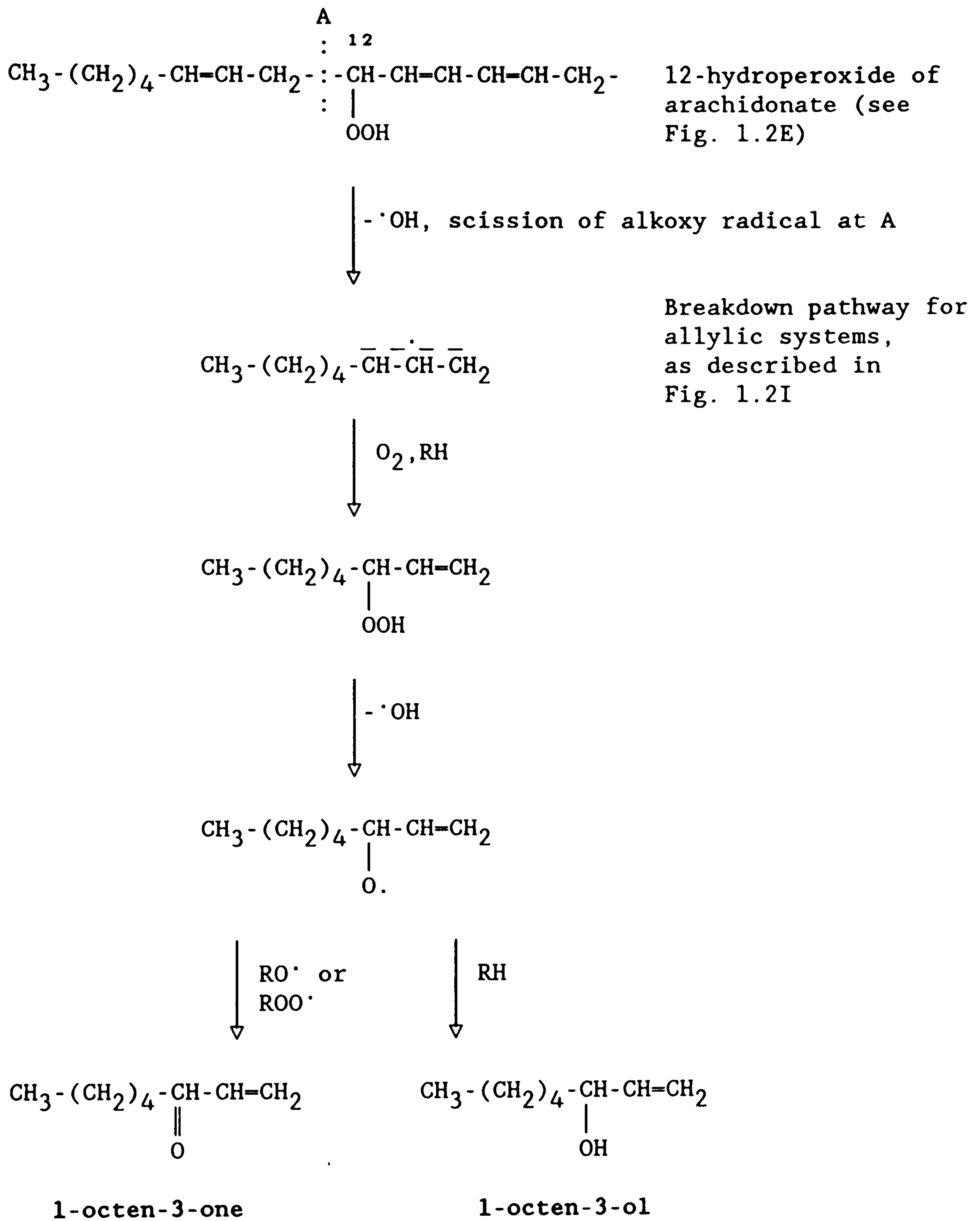
The odour thresholds in water of saturated ketones are in the region of 1 part in 10^6 or 10^7, which is considerably higher than those of the isomeric aldehydes. However, 1-alken-3-ones are much more potent; 1-octen-3-one has an odour threshold of *ca* 1 part in 10^{10} (Grosch 1982). 2-Alkanones often possess "fruity" or floral" aromas, becoming more "oily" with increasing chain length. Vinyl ketones possess a range of unpleasant odours and tend to be lachrymatory at high concentrations (Forss 1972).

Ketoaldehydes (eg. pyruvaldehyde) and diketones (eg. 2,3-butanedione) can arise not only from the thermal breakdown of sugars, but also from lipid oxidation (Forss 1972). These compounds are reactive participants in other flavour forming reactions, for instance Strecker degradation.

1.2.4.3 Alcohols

The occurrence of both saturated and unsaturated alcohols in foods is widespread. However, the flavour thresholds of alcohols are generally higher than their carbonyl analogues and these compounds have consequently been considered of lesser importance to flavour and aroma. The flavour thresholds of the saturated alcohols range from 3 parts in 10^7 to 2 parts in 10^4 but are mainly in the order of 1 part in 10^6 (Forss 1972). Unsaturated alcohols have lower thresholds down to 1 parts in 10^9 (Forss 1972) and many have

Figure 1.2L Formation of 1-octen-3-one and 1-octen-3-ol from arachidonic esters (Wilkinson & Stark 1967).



distinctive odours, such as those reminiscent of "mushroom" (1-octen-3-ol) and "green leaf" (2-hexen-1-ol and 3-hexen-1-ol).

The degradation of lipid hydroperoxides frequently gives rise to alcohols via the formation of alkyl or alkenyl radicals, which undergo oxidation to give primary hydroperoxides; these form alkoxy radicals and thence the corresponding alcohols (including 1-alkanols, 2-alkanols, 1-alken-3-ols); see Sec. 1.2.1.2, Figs. 1.2G-J. Mechanisms for the formation of specific alkenols (1-octen-3-ol, 1-penten-3-ol and 3-penten-2-ol) have been proposed from various polyunsaturated fatty acid esters (Forss 1972).

1.2.4.4 Aromatic compounds

Although most of the products of lipid oxidation are aliphatic, a few aromatic compounds are formed by cyclization of the more unsaturated lipid breakdown products. Such compounds include benzaldehyde and benzoic acid, alkylbenzenes, acetophenone, various phenylketones, phenylaldehydes and 2-alkylfurans (Forss 1972). Such compounds have also been identified in a range of foods; 2-pentylfuran is a constituent of many foods, including chicken, coffee and soybean oil; in this latter it contributes a "beany" flavour. Various naphthalenes have been identified in roasted peanuts and alkylbenzenes occur in cooked chicken (Nonaka *et al* 1967; Forss 1972); these compounds have also been attributed to lipid oxidation.

Benzaldehyde and acetophenone have odour thresholds in the region of 1 part in 10^9 and 7 parts in 10^5 (Stahl 1973) and are described as "bitter, almond" and "sweet, pungent, acacia", respectively (Furia and Bellanca 1975). Benzoic acid has little odour but exhibits a "sweet-sour, acrid" taste and has a flavour threshold of 8 parts in 10^5. A mechanism for the formation of benzaldehyde and benzoic acid by free radical oxidation of linoleic acid has been suggested (Kawada *et al* 1967). However, Bruechert *et al* (1988) have found that benzaldehyde is formed during the thermal degradation of 2,4-decadienal and that radical-trapping antioxidants do not inhibit the reaction. They propose that benzaldehyde can be formed by a retro-aldol degradation reaction between 2,4-decadienal and hexanal.

2-Alkylfurans can be formed from the hydroperoxides of polyunsaturated fatty acids (Sec.1.2.1.2, Fig. 1.2J) or directly from alkadienals by cyclization. The odour thresholds of the 2-alkylfurans are in the order of 1 part in 10^5 or 10^6 and their odours tend to be "sweet"; 2-pentylfuran is also described as "pungent" and "fruity" (Fors 1983).

1.2.4.5 Acids and esters

Both saturated and unsaturated acids (C_1 to C_{20}) are common constituents of foods and in some cases contribute to the overall flavour. Saturated fatty acids (especially butyric acid) are key flavour components of butter and cheese, while many short chain unsaturated acids contribute to the odours of plants and fruits; for instance, 2-methyl-2-pentenoic acid is thought to be an important component of strawberry flavour (Forss 1972).

Acids may be formed by hydrolysis of triglycerides, thermal degradation reactions, oxidative breakdown of fatty acids or by autoxidation of aldehydes or ketones (Forss 1972). Ketoacids are products of lipid oxidation and are themselves precursors of 2-alkanones and lactones respectively.

The flavour thresholds of saturated acids (C_4 to C_{10}) are mostly in the range of 5 parts in 10^6 (Forss 1972); many have "rancid" or "oily" odours and taste unpleasantly "acrid" or "sour" (Furia and Bellanca 1975). The odour thresholds of these compounds depend upon the pH of the medium as only undissociated molecules are volatile (Grosch 1982). Long chain fatty acids (C_{18} onwards) are odourless (Forss 1972).

Aliphatic esters are reponsible for the characteristic flavours of cheese and many fruits. They are thought to be formed by enzyme activity in fruit, while in cheese other mechanisms, such as free radical pathways, are thought to pertain (Forss 1972). Their flavour thresholds range down to 1 part in 10^8.

1.2.4.6 Lactones

γ-Lactones (dihydro-2(5H)-furanones) and δ-lactones (tetrahydro-2-pyranones) are commonly found in foods and long-chain lactones are derived from lipids by both enzymic and thermal degradation mechanisms (Forss 1972). δ-Lactones have been isolated from both dairy products and coconut oil, while γ-lactones are more prevalent in peaches, apricots and other fruits as well as in autoxidatized cottonseed and soybean oils. Unsaturated alkenolactones have been identified in fried triolein, corn oil and various vegetable sources (Forss 1972). The presence of saturated γ- and δ-lactones have been reported in animal fats (Watanabe and Sato 1968).

Lactones may be formed from the 4- or 5-hydroxy alkanoic acids (or their triglyceride esters) which can cyclize to give γ- and δ-lactones respectively (Forss 1972). They have a range of flavours, with γ-lactones possessing predominantly "herbaceous", "fruity" and "nutty" flavours and many δ-lactones being described as "creamy", "buttery" and "fruity". The flavour thresholds of lipid-derived saturated γ- and δ-lactones range from 3 parts in 10^5 down to 1 part in 10^7 or less (Forss 1972; Grosch 1982).

1.2.4.7 Hydrocarbons

Alkanes and alkenes are readily formed by the mechanisms of autoxidation and are volatile components of many foods. However, the high odour thresholds of alkanes mean that these compounds are of very little importance in aroma or flavour; these range from *ca* 1 part in 10^4 (hexane) to values in excess of 1 part in 100 (Forss 1972). Some alkenes and alkynes are more potent with odour thresholds down to 1 part in 10^6. It has been suggested that it is unsaturated hydrocarbons rather than carbonyl compounds which are responsible for off-odours in irradiated fats (Merritt *et al* 1967).

1.3 LIPIDS AND THE MAILLARD REACTION

Lipid oxidation and the Maillard reaction between amino acids and reducing sugars are two of the most important reactions for the formation of odours in cooked foods. The previous Sections of this Introduction have presented the major pathways of these reactions and have described how they contribute to the volatile aroma compounds derived from foods. However, all foods, whether animal or vegetable in origin, consist of a complex mixture of components. Thus, it is rare in normal foods for either of these reactions to occur in isolation from the other and it may be expected that the course of each reaction would be modified by the reactants, intermediates and products of the other.

Interest in the effect of the Maillard reaction on lipid oxidation (and vice versa) has largely centred round reactions causing deterioration of food quality and their amelioration. Lipid oxidation products not only cause rancidity themselves, but also induce various deteriorative reactions in proteins, amino acids and other food components. In contrast, Maillard reaction products can exert an antioxidative effect and may be used to protect food lipids from oxidation. Only recently has the interaction between these two reactions been implicated in the formation of the desirable flavour of food.

1.3.1 DETERIORATION REACTIONS OCCURRING BETWEEN LIPIDS AND MAILLARD REACTANTS.

Peroxidizing lipids can cause damage to other cellular components including proteins, amino acids and nucleotides (Roubal and Tappel 1966a, 1967; Machlin and Bendich 1987). Such reactions have important consequences for living organisms both when they occur *in vivo* and in foodstuffs. Peroxidation of lipids *in vivo* may cause the development of 'aging pigments', cross-linking of proteins and damage to cell membranes and has been cited as a possible cause of numerous human disorders, including increased membrane permeability, alcohol-induced liver damage, aging, cardiovascular disease and the action of carcinogens (Karel *et al*

1975; Machlin and Bendich 1987). However, healthy living tissue is generally immune to such degradative reactions, due to the highly organised cellular compartmentalisation which keeps separate those cell components which would otherwise damage each other (Gardner 1979). However, once a tissue is disrupted (as may occur on slaughter of food animals or during food production or preparation) this precise structure is destroyed, inducing the onset of lipid oxidation and permitting the products of this process to react with other constituents of the cell. Thus, in foods, peroxidizing lipids may induce the formation of toxic or otherwise biologically active compounds (Karel 1973).

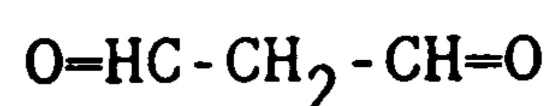
Under oxidizing conditions, proteins and peroxidized lipid tend to aggregate into complexes through hydrophobic association or hydrogen-bonding (Gardner 1979). In the opinion of Karel (1973), non-specific hydrophobic association is of primary importance under these conditions with hydrogen-bonding playing a secondary role. This is because, in aqueous solution, the strength of water-water hydrogen bonds greatly exceeds that of the attraction between water and the non-polar regions of proteins and lipids. Although bonding between these areas of lipids and proteins is dependent largely upon relatively weak Van der Waals forces, the excluding effect of water results in these species being 'pushed together', increasing the attraction between the non-polar groups; the resulting association is referred to as 'hydrophobic bonding' or 'hydrophobic association'. Various workers have demonstrated that oxidized lipids (Narayan *et al* 1964), fatty acids and their hydroperoxides (Matsushita *et al* 1970) and products of oxidation such as aldehydes, ketones and alcohols (Beyeler and Solms 1974) can bind to protein by such weak, non-specific forces.

The formation of such complexes can cause protein damage in several ways. Lipids and their oxidation products can inactivate enzymes by occluding the hydrophobic areas of the protein and thus may block the active sites (Gardner 1979). These compounds can usually be removed from the protein by extraction with a suitable solvent. However, the close proximity of peroxidized lipids and proteins in such complexes can promote further reactions which may irreversibly alter the structure of the proteins. These include the reactions of lipid-derived carbonyl compounds with protein amino groups and the formation of protein free radicals.

1.3.1.1 Reaction of carbonyl compounds with protein amino groups

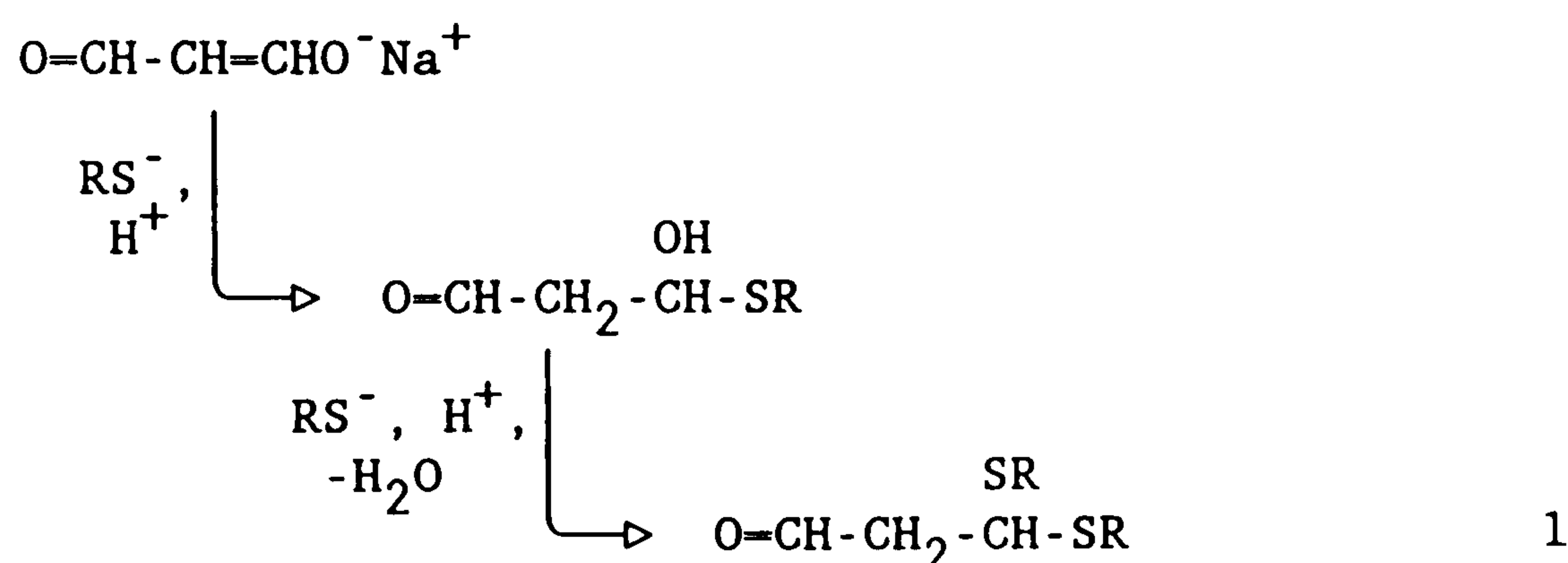
Carbonyl compounds formed by lipid oxidation can react with protein amino groups to give Schiff base-type products. Pokorny *et al* (1981) attributes the browning of fish during frozen storage to such reactions and proposes that the Schiff base intermediates further react by aldolization reactions to give polymeric brown pigments. Such reactions alter the sensory and nutritive properties of the fish. Yanagita and Sugano (1974) showed that, when ethyl linoleate was autoxidized in the presence of egg albumin, increasing browning corresponded to a decrease in the levels of available lysine. Unsaturated carbonyl compounds have been found to react with proteins more readily than the saturated compounds (Fujimoto *et al* 1968) and dicarbonyls are especially reactive due to their participation in Strecker degradation reactions (Velisek *et al* 1972). Ketones were relatively inactive, with the exception of hydroxy ketones which react as rapidly as aldehydes (Pokorny *et al* 1981).

The reaction of one particular aldehyde has been extensively studied. Malonaldehyde (or malondialdehyde) is a product of the oxidation of linolenic, arachidonic and other fatty acids, especially those with three or more double bonds (Gardner 1979) and its detection using the 2-thiobarbituric acid (TBA) test is widely used as a method for determining the extent of lipid oxidation.

 malonaldehyde

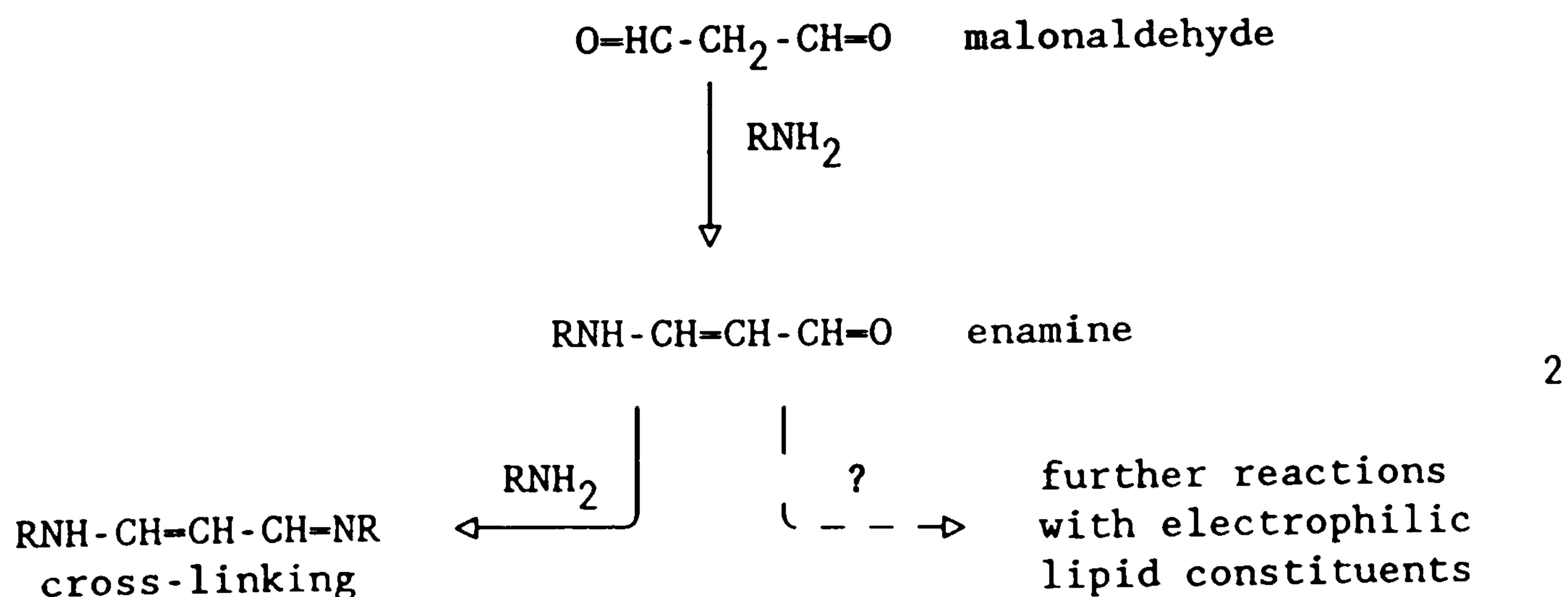
In *vivo* reactions between malonaldehyde and proteins or amino acids have been implicated in the formation of the so-called 'aging pigment' and in the cross-linking of proteins. The *in vitro* incubation of malonaldehyde with albumin from bovine plasma results in the binding of the aldehyde to the protein and loss of available lysine ϵ-amino groups (Crawford *et al* 1967). Buttkus (1967) also found lysine to be the most labile of the amino acids in myosin; the ϵ-amino groups of lysine are attacked even in the frozen state.

Other sites of reaction with malonaldehyde appear to be the amino acids, histidine, tyrosine, arginine and also methionine and cysteine (Buttkus 1967). In the reaction of the amino acid, cysteine, with malonaldehyde, evidence suggests that the thiol group as well as the amino group undergoes reaction (Buttkus 1969). The suggested mechanism for the reaction of the thiol group (at pH 7) involves addition to the enolic bond of malonaldehyde as shown (1).



In contrast, methional reacted with malonaldehyde only at the *α*-amino group, in a similar manner to glycine (Buttkus 1969).

The bifunctional nature of malonaldehyde facilitates the cross-linking of proteins, via the formation of conjugated Schiff bases (Andrews *et al* 1965; Chio and Tappel 1969; Kikugawa and Beppu 1987). In addition, the reaction of malonaldehyde with protein amino groups would be expected to yield enamines, as shown in reaction 2, unlike most carbonyl compounds which would give the imine. Such enamine derivatives may be capable of undergoing further reactions with lipid constituents (Crawford *et al* 1967).



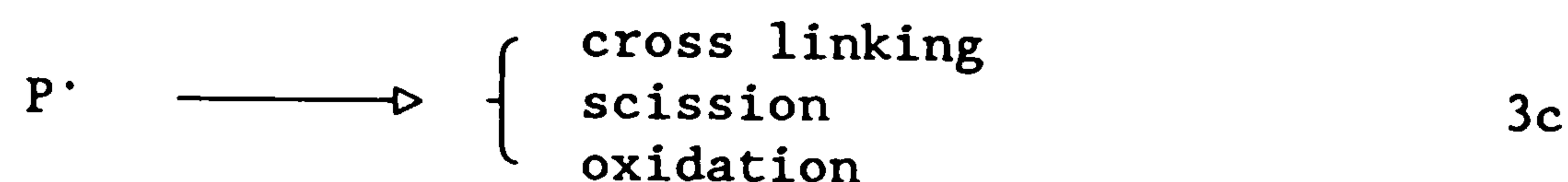
Monofunctional aldehydes, such as alkanals, 2-alkenals and 2,4-alkadienals have also been implicated in the formation of protein cross-links (Kikugawa and Beppu 1987). It is suggested that the Schiff bases arising from reaction with amino groups are then subject to nucleophilic attack by protein thiol groups.

1.3.1.2 Formation of protein free radicals

Attack by peroxidizing lipids has been held responsible for the polymerization of proteins, enzymes and nucleotides and the destruction of amino acids (Roubal and Tappel 1966a,b, 1967). Such damage is similar in form and severity to that induced by ionizing radiation which, like lipid peroxidation, leads to the generation of free radicals (Desai and Tappel 1963; Roubal and Tappel 1966a; Chio and Tappel 1969). The formation of lipid-protein complexes allows the intimate contact necessary for the transfer of free radicals between these species (Gardner 1979).

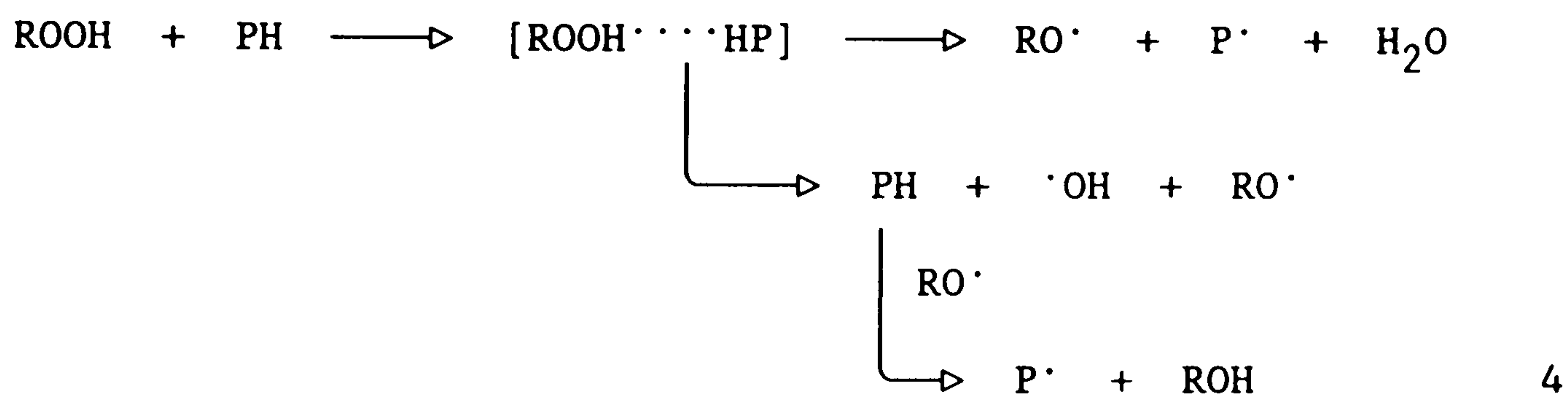
Evidence for the formation of protein radicals *per se* was obtained using electron spin resonance (ESR; also known as electron paramagnetic resonance) by Roubal (1970). It was deduced that radicals derived from lipid oxidation were 'trapped' by the protein matrix and that these radicals may be reponsible for the observed damage to proteins and amino acids. Schaich and Karel (1975, 1976) studied the formation of free radicals in proteins and amino acids in the presence of peroxidizing methyl linoleate and found that increased lipid oxidation results in greater formation of protein radicals. The accumulation of protein radicals appears to be concomitant with the build up of lipid hydroperoxides, with most formation occurring as the hydroperoxides begin to break down (Schaich and Karel 1975).

The generation of protein radicals is much accelerated by the presence of metal ions, haem compounds, etc, which catalyse the breakdown of hydroperoxides to give $ROO^{\cdot}$ and $RO^{\cdot}$ (Gardner 1979); see reactions 6a and b, Section 1.2.1.1. It has been suggested that these species react with proteins to generate protein radicals as shown in reactions 3a and b (Schaich and Karel 1976).



In dry model systems, alkoxy radicals appear to be most effective at inducing the formation of protein radicals, although peroxy radicals may also contribute. Close contact between lipid and protein appears to be crucial for direct hydrogen abstraction from protein sites by such radicals (Schaich and Karel 1976). Further reactions such as cross-linking, scission or oxidation may damage the structure of the protein (reaction 3c; Gardner 1979).

Free radical transfer may occur via the formation of complexes between lipid hydroperoxides and N or S groups of amino acid residues. Such reactions could initiate further free radical chain reactions, as illustrated in reaction sequence 4 (Schaich and Karel 1976).



Protein free radicals give ESR signals corresponding to radicals on the α-sidechains and thiol groups of the component amino acids of a protein. The range of ESR signals observed suggests that radicals are formed on a number of different amino acids. Although direct hydrogen abstraction can only occur at the protein-lipid interface, intramolecular migration may allow transfer of radicals from the site of attack to other regions of the protein (Schaich and Karel 1976).

Studies on the formation of free radicals in individual amino acids (Schaich and Karel 1976) have indicated that, of eighteen amino acids studied, four non-sulphur amino acids (lysine, arginine, histidine and tryptophan) form detectable amounts of radicals with oxidizing methyl linoleate. A mechanism has since

been suggested for the free radical breakdown of tryptophan (Yong *et al* 1980). Of the sulphur-containing amino acids, only cysteine forms radicals readily; cystine reacts slowly and methionine not at all (Schaich and Karel 1976). Studies on proteins suggest that lipid peroxides and/or free radicals can readily abstract hydrogen from protein thiol groups but do not easily break disulphide bonds (Karel *et al* 1975). Such reports substantiate claims that free radicals are an important cause of lipid peroxide damage to proteins in general and to sulphydryl proteins in particular (Schaich and Karel 1976). Sulphydryl enzymes are known to be more susceptible to inactivation by lipids than non-sulphydryl enzymes (Chio and Tappel 1969).

Gardner (1976) cites evidence for the formation of bonds between the S of cysteine and the double bond system of linoleic acid alkoxy radicals in the absence of oxygen. It is suggested that addition of cysteine to the conjugated diene by the anti-Markownikoff rule and formation of the disulphide indicate that thiyl radicals are involved. The presence of Fe(III) ions was essential for reaction and it is presumed that the formation of iron-cysteine complexes allows an electron shift to give Fe(II) and the thiyl radical. In the presence of oxygen, such amino acid-lipid adducts are replaced by various oxygenated products.

The main products of the peroxide-induced oxidation of cysteine are cystine, cystine monoxide and cystine dioxide (Finlay and Lundin 1980). Cleavage of the thiol group can also release H_2S to leave alanine (Roubal and Tappel 1966a). The oxidation of cysteine can occur without metal catalysts, but is much faster in the presence of Cu^{2+} or Fe^{2+} ions (Jocelyn 1972) and appears to increase with a rise in pH (Finlay and Lundin 1980).

Most of these studies were conducted under anhydrous conditions to give sufficiently stable radicals for detailed study. Comparison with aqueous systems has shown that water decreases the damage to proteins (Zirlin and Karel 1969) and has a quenching effect on the formation of protein radicals; a water activity of 0.75 gave about one quarter of the concentration of protein radicals derived from anhydrous systems (Schaich and Karel 1975). It was suggested that water may influence lipid-protein free radical interactions by depressing the concentrations of initiating radicals and by

affecting the mobility of reactants, thus altering the balance between free radical transfer and termination reactions.

1.3.1.3 Other lipid-Maillard deteriorative reactions

The degradation of proteins and amino acids by lipid hydroperoxides is not the only type of degradative reaction which occurs between lipids and Maillard reactants. Deteriorative reactions in various commodities have been attributed to Maillard-type reactions between the amino groups of certain phospholipid polar moieties and carbonyl compounds. The browning and off-flavours which occur in dried egg have long been thought to arise from the reaction between such amino groups and glucose (Lea 1957).

Black *et al* (1978) found that the commercial treatment of corn with ammonia (to control moulds) caused a reduction in the content of linoleic acid. Their results indicate that ammonia reacts chemically with the double bonds of the polyunsaturated fatty acids of the corn triglycerides. The product is a nitrogen-containing triglyceride fraction, in which the reacted fatty acid moieties are still attached to the glycerol chain. Ammonia is commonly formed as a product of the Maillard reaction; in particular, the Strecker degradation of cysteine with dicarbonyl compounds generates significant quantities of ammonia. Thus, the degradation of lipids by the action of ammonia may be important both in heated foods and in the study of model Maillard systems.

1.3.2 ANTIOXIDATIVE EFFECT OF MAILLARD REACTION PRODUCTS

Not only does the presence of lipid affect the structure and stability of proteins and amino acids, but the presence of amino acids and sugars and their reaction products is known to affect the oxidation of lipids. Maillard reaction products were first demonstrated to possess an antioxidative effect by Franzke and Iwainsky (1954), who showed that the products of the reaction of glycine or monosodium glutamate with glucose improved the stability of margarine. Griffith and Johnson (1957) found that the

inclusion of dextrose in cookie dough not only increased browning but also increased oxidative stability during storage. Since then, a great deal of interest has been shown in this property as it offers the possibility of using natural food components as antioxidants.

Castell (1971) studied the unusual resistance of the highly unsaturated lipids in lean fish. When extracted from the tissue they developed the rancid odours typical of oxidized fish oils; however, when remaining in close contact with the muscle protein, they were resistant to oxidation for long periods.

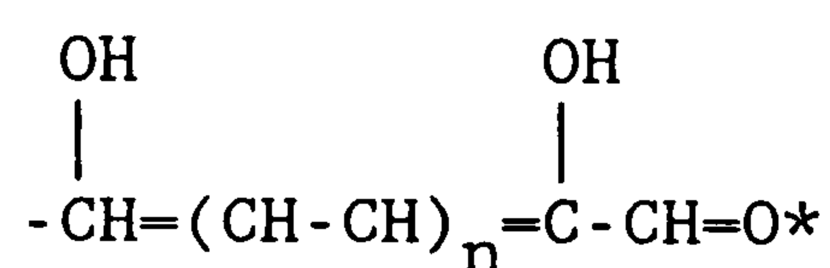
Heating meat to very high temperatures has been found to cause the generation of unknown substances with antioxidant activity, which are capable of retarding lipid oxidation (Zipser and Watts 1961). The study of such substances by Sato *et al* (1973) showed that they occur in diffusates from beef, pork and turkey only after extreme heating and do not act by removing iron or ascorbic acid catalysts. It was suggested that the inhibitory properties of these diffusates are caused by products of reactions between sugars and amino acids or proteins. Solutions of heated (but not unheated) Maillard model reactions were shown to reduce 'warmed over flavour' in meat. The suppression of 'warmed over flavour' by Maillard reaction products has been the subject of recent reviews (Bailey *et al* 1987; Bailey 1988); the products from heated glucose and histidine have proved effective inhibitors of oxidation in cooked meats.

In general, the practical use of Maillard reaction products as antioxidants is limited by their brown colour, poor lipid solubility and flavour characteristics; for these reasons, they are unsuitable for use in pure oils. However, some workers have investigated the viability of using commercially available protein and carbohydrate sources to protect various food products against oxidative rancidity. The use of protein hydrolysates and glucose proved effective as antioxidants in sausages and cookies (Lingnert and Eriksson 1981; Lingnert and Hall 1986), while blood and starch hydrolysates were efficient antioxidants in oil-water emulsion-containing products, e.g. sauces, custards (Obretenov *et al* 1986).

1.3.2.1 Maillard products responsible for antioxidative activity

The question of which of the many products of the Maillard reaction are responsible for the observed antioxidative effect has been studied for many years and has still not been fully resolved. Griffith and Johnson (1957) suggested that reductones were responsible for the antioxidative effects and this hypothesis was supported by work involving the isolation and study of reductones.

Reductones are resonance-stabilized enediols possessing the general formula:



The unsaturation shown at the askerisk is usually that of a carbonyl group (Hodge 1953). Reductones are strong reducing agents in acid, neutral and alkali media. Such compounds include ascorbic acid, dihydroxy maleic acid and reductic acid (Evans *et al* 1958).

Evans *et al* (1958) studied amino-hexose reductones, from the reaction of secondary amines with hexose sugars, and showed that these species increase the induction period and decrease oxygen absorption and peroxide formation when present in concentrations from 0.2% down to 0.001%. Various other reductones and low molecular weight Schiff bases have also been shown to exert an antioxidative effect on soybean and cottonseed oils (Obata *et al* 1971).

In contrast, other workers have investigated the antioxidative effect of a number of reductones and found that, although some showed antioxidative activity under dry conditions, no such activity was observed in aqueous systems. However, the melanoidin fractions demonstrated considerable antioxidative activity (Yamaguchi and Koyama 1967; Yamaguchi and Fujimaki 1970; Lingnert and Eriksson 1981).

Efforts to establish precisely which compounds confer antioxidative activity have included the fractionation of products of the reaction between glycine and xylose according to molecular weight (Yamaguchi *et al* 1981). The antioxidative activity of each fraction was tested in an aqueous/ethanolic solution of linoleic

acid. The coloured, high molecular weight melanoidin fraction was found to possess high antioxidative activity, while the fraction containing low molecular weight reductones did not. Further fractionation showed that, although all coloured fractions possessed some antioxidative activity, one particular fraction obtained by gel filtration, of molecular weight ca. 4500, proved comparable in efficiency to several commercially used antioxidants, eg. BHT, BHA. These results are consistent with those of Lingnert *et al* (1983) who found that the active antioxidant among the products of the reaction between histidine and glucose is a fraction of molecular weight between 1000 and 10000. Antioxidative activity is retained following the breakdown of the melanoidin by ozone oxidation to reduce the colour (Yamaguchi 1986).

Eichner (1981) has reported that in systems with low water content the colourless intermediates of the Maillard reaction are the main cause of antioxidative effects. Low water activities not only inhibit browning (Eichner 1981), but also favour the antioxidative activity of reductones (Lingnert and Eriksson 1981). At low water activity, the colourless intermediates formed from the reaction of glucose and lysine demonstrated a clear antioxidative effect on the autoxidation of methyl linoleate (Eichner 1981). However, when the water content was increased and browning permitted, the antioxidative activity also increased, together with the reducing power of the Maillard reaction products. These results seem to suggest that melanoidins may be responsible for antioxidative activity exerted in aqueous systems, while low molecular weight intermediates become more important with increasing dehydration. In contrast, most of the antioxidative activity obtained from an aqueous 1:1 mixture of arginine and xylose was lost upon dialysis and, therefore, must be due to low molecular weight compounds (Waller *et al* 1983).

The relationship between melanoidin formation and antioxidative effect is thrown into question by the studies of Lingnert and Eriksson (1981) on the reaction of various amino acid + sugar combinations in aqueous solution; little correlation between colour formation and antioxidative effect was observed . In addition, increasing the proportion of the amino acid (histidine) had little effect on colour but did increase antioxidative activity. Thus, it was concluded that the reaction paths important

for the formation of antioxidative compounds are different to those responsible for colour formation. Other workers (Hwang and Kim 1973; Rhee and Kim 1975) also found no correlation between the development of antioxidative effect and colour formation for either Maillard or caramelization reactions conducted under aqueous conditions; most of the antioxidative activity developed early in the reaction before colour formation had become significant. Thus, both low molecular weight reductones and the polymeric melanoidins appear to exert antioxidative effects under different conditions, but the relationship between this effect and colour formation remains contentious.

1.3.2.2 Mechanisms responsible for antioxidative activity

The mechanisms by which Maillard reaction products exert an antioxidative effect are still not clear, but several suggestions have been made. A comparison of the relative antioxidative effects of various amino acid + sugar combinations suggested that the greatest antioxidative effect is observed in the presence of the basic amino acids, histidine, arginine and lysine, while other amino acids, such as glutamic acid, valine and cysteine, show much smaller effects (Lingnert and Eriksson 1981). It has been shown that basic amino acid groups themselves are antioxidative under dry conditions (Koch *et al* 1971) or at alkali pH, when the amino groups exist largely in the non-ionized form (Eichner 1981); under acidic conditions no antioxidative effect is observed. The addition of EDTA to the reaction mixtures does not modify the degree of oxidation observed; thus, it does not appear that the amino groups are acting by complexing catalytic metal ions. It is unlikely that the antioxidative effect observed in the studies of Lingnert and Eriksson (1981) is caused by the amino groups of the basic amino acid as there is no alteration in antioxidative effect over a range of initial pH values from pH 11 to 3.

Eichner (1981) suggests that the Maillard reaction intermediates exerting an antioxidative effect are 1,2-enaminols, derived from Amadori rearrangements; such intermediates may reduce peroxides and other lipid radicals to give hydroxy linoleic acid, which does not undergo further decomposition to give rancid products. Thus, Maillard products may exert an antioxidative effect by terminating

free radical reactions. The extent of hydroperoxide removal appears to be linked to the reducing power of the model system and not to colour intensity.

Lingnert *et al* (1983) examined a coloured, high molecular weight fraction of the products from the reaction between histidine and glucose with a strong antioxidative effect. The analytical composition of this fraction is cited as C (54.3%): H (5.4%): N (12.6%): O (24.5%). These data would give a molecular formula of approx. $n(C_{15}H_{18}N_3O_5)$; calculation of the number of 'double bond equivalents' indicates that this compound has a very high degree of unsaturation (8 or 9 double bonds/ring closures per 15 carbons) and would be consistent with a highly aromatic structure. Studies using electron spin resonance (ESR), showed that the melanoidin fraction contains stable free radicals and that there is some tendency for the antioxidative effect to be related to the size of the ESR signal (Lingnert *et al* 1983). Stable free radicals have also been detected in the melanoidin fraction obtained from the reaction of glucose with 4-chloroaniline (Lessig and Baltes 1981); this fraction was found to consist largely of aromatic structural units. Such stable free radicals may interact with the radicals of the lipid oxidation chain, inhibiting oxidation in the manner of a Type I 'free radical chain stopper' antioxidant (see Section 1.2.1.1).

A possible explanation for the diversity of compounds and fractions showing antioxidative activity is suggested by Lingnert and Eriksson (1981); the actual antioxidative compound may be a relatively small molecule, possibly a free radical, which is strongly adsorbed to a wide range of Maillard reaction products, and is therefore difficult to isolate. Such a possibility would be consistent with the results of Yamaguchi (1986) that break up of melanoidins using ozone oxidation does not destroy antioxidative activity.

Although the evidence is sometimes contradictory, it appears that an antioxidative effect may be exerted by a range of Maillard reaction products; high molecular weight melanoidins have most activity under aqueous conditions while low molecular weight reductones or analogous compounds are more effective at low water activities. There is evidence that at least some of these compounds exert their effect by free radical mechanisms, although

redox reactions and inhibition of pro-oxidant metals may also play a part.

1.3.2.3 Effect of unheated Maillard reactants

While the products of the Maillard reaction have been shown to exert antioxidative activity, the effect on lipid oxidation of amino acids and sugars themselves is less clear. Farag *et al* (1978a,b,c) have investigated the effect of several amino acids on the oxidation of linoleic acid and cottonseed oil at 40 ^{o}C. In freeze-dried model systems, alanine, serine, phenylalanine, tryptophan and histidine all exhibited a small antioxidative effect (Farag *et al* 1978a), while, in an aqueous emulsion, the same amino acids showed a pro-oxidative capacity (Farag *et al* 1978b). It was suggested that in the aqueous system, the presence of protonated amino groups in the surface of the lipid mycelles may be responsible for catalysing lipid oxidation, while in non-aqueous systems the trace quantities of water are insufficient to form protonated amino groups. Similar results were obtained by Riisom *et al* (1980). Cysteine behaves differently to the other amino acids tested and exerts a pro-oxidative effect, under aqueous or non-aqueous conditions. This effect exceeds that for the other amino acids and is concomitant with rapid destruction of the thiol group (Farag *et al* 1978c).

Studies on the effect of sugars on lipid oxidation have shown that oxidative stability improves with increasing sugar content (Sims *et al* 1979). This finding is attributed to physical factors rather than to chemical effects. The inhibition of oxidation in aqueous emulsions may be due to increasing viscosity and a lower concentration of oxygen, while in freeze-dried systems the dispersion of oil droplets and the porosity of the particles to oxygen is thought to dictate whether an anti- or pro-oxidative effect prevails.

1.3.3 VOLATILE PRODUCTS FROM LIPID-MAILLARD INTERACTIONS IN MODEL SYSTEMS

Evidence for reactions between the products of lipid oxidation and the Maillard reaction has been obtained using model systems designed to investigate specific aspects of this interaction. Such evidence generally comprises the identification of products whose mechanism of formation is difficult otherwise to explain: for instance, long-chain heterocyclic compounds or aliphatic compounds incorporating a heteroatom from the amino acid.

Montgomery and Day (1965) reacted n-heptanal with the ethyl ester of tyrosine or glycine. They deduced that the aldehyde reacts with the amino groups of the amino acid to yield an imine. Condensation reactions with further molecules of aldehyde gave non-N-containing polymeric pigments and 2-alkenals; thus, the amino acid was thought to serve as a catalyst in aldol condensation reactions of the aldehydes. Such reactions may be responsible for depletion of the carbonyl flavour compounds in foods. Burton *et al* (1963) commented that, of various carbonyl compounds, α,β-unsaturated aldehydes are most reactive in reactions with glycine.

A number of volatile compounds obtained from the amino acid, valine, and the triglyceride, tricaproin, when these compounds were heated individually could no longer be detected when they were heated together (Lien and Nawar 1974b). Instead, caproic nitrile and two amides were identified, presumed to be formed by the reaction of caproic acid or its anhydride with ammonia or amines formed from the degradation of valine. Sims and Fioriti (1975) also identified amides as products of high temperature reactions between fatty esters and a number of amino acids.

More recently, the use of capillary GC has permitted the identification of an extended range of volatile products from reactions between amino acids and fats or fatty acids. The thermal reaction of linoleic acid or its esters with valine gave many products specific to the interaction between the two species (Henderson and Nawar 1981). Predominant amongst these were a series of 2-alkylpyridines, with 2-pentylpyridine the most abundant. It was proposed that this compound arose from the reaction of 2,4-decadienal with ammonia as suggested by Buttery *et*

al (1977); see Figure 2.3L, Section 2.3.1. Also formed as 'interaction products' were several 2-alkylpyrroles; it was suggested that these compounds were either formed from 2-alkenals and ammonia (Fig. 1.3B) or by the reaction of 1,3-butadiene and ammonia to give pyrrole, followed by reaction with alkoxides (Henderson and Nawar 1981).

The presence of an additional amino group in lysine gives rise to 3- and 4-alkylpyridines as well as 2-alkylpyridines, when reacted with tricaproin or tributyrin (Breitbart and Nawar 1981). These compounds are thought to arise from the reaction of ammonia with certain carbonyl compounds (butanal, acetaldehyde, acetone) arising from the degradation of lysine. 2-Butylpyridine and 2-pentylpyridine were identified amongst the numerous volatile compounds obtained from beef fat heated with glycine (Ohnishi and Shibamoto 1984). These authors suggest that these pyridines arise from the reaction of n-aldehydes with ammonia. A possible mechanism for such a reaction is that suggested by Suyama and Adachi (1980), which is thought to involve the condensation of three molecules of propanal with one of glycine (see Fig. 1.10, Sec. 1.1.1.3). Similar condensation reactions could explain the generation of tri-alkylpyridines with propyl, butyl and pentyl substituents from reactions between pentanal or hexanal and ammonium sulphide (Hwang *et al* 1986).

The above publications did not report the presence of any pyrazines, presumably due to the lack of suitable α-dicarbonyl or α-hydroxycarbonyl compounds to undergo Strecker degradation reactions. However, the volatile products of the reaction between zein, starch and oil from corn include several long-chain pyrazines which were deduced to be products from the interaction of lipid, protein and carbohydrate (Huang *et al* 1987; Bruechert *et al* 1988; Ho *et al* 1989). The authors propose that the mechanism of formation of these compounds involves the reaction of pentanal (from lipid oxidation) with Strecker degradation products from the reaction between the protein and carbohydrate (Huang *et al* 1987).

Very recently, Chiu *et al* (1990) has identified various long-chain pyrazines from the reaction of acetol and ammonium acetate with pentanal or hexanal. He proposes that acetol reacts with ammonia to give the aminoketone, from which the dihydrodimethylpyrazine is formed by autocondensation; this compound may subsequently react

with pentanal (for example) by a condensation reaction to give a pentyldimethylpyrazine (see Fig. 1.3A).

The presence of sulphur-containing amino acids in reactions between Maillard reactants and lipids may yield a range of long chain sulphur-containing heterocyclic compounds. The thermal decomposition of cysteine with tributyrin gives a number of products from the interaction of these two species, including 2-propyl thiazoline and 2-propylthiazole (Severin and Ledl 1972). The proposed mechanism of formation involves the reaction of butyric acid with the amino group of cysteine to form an amide which then cyclizes.

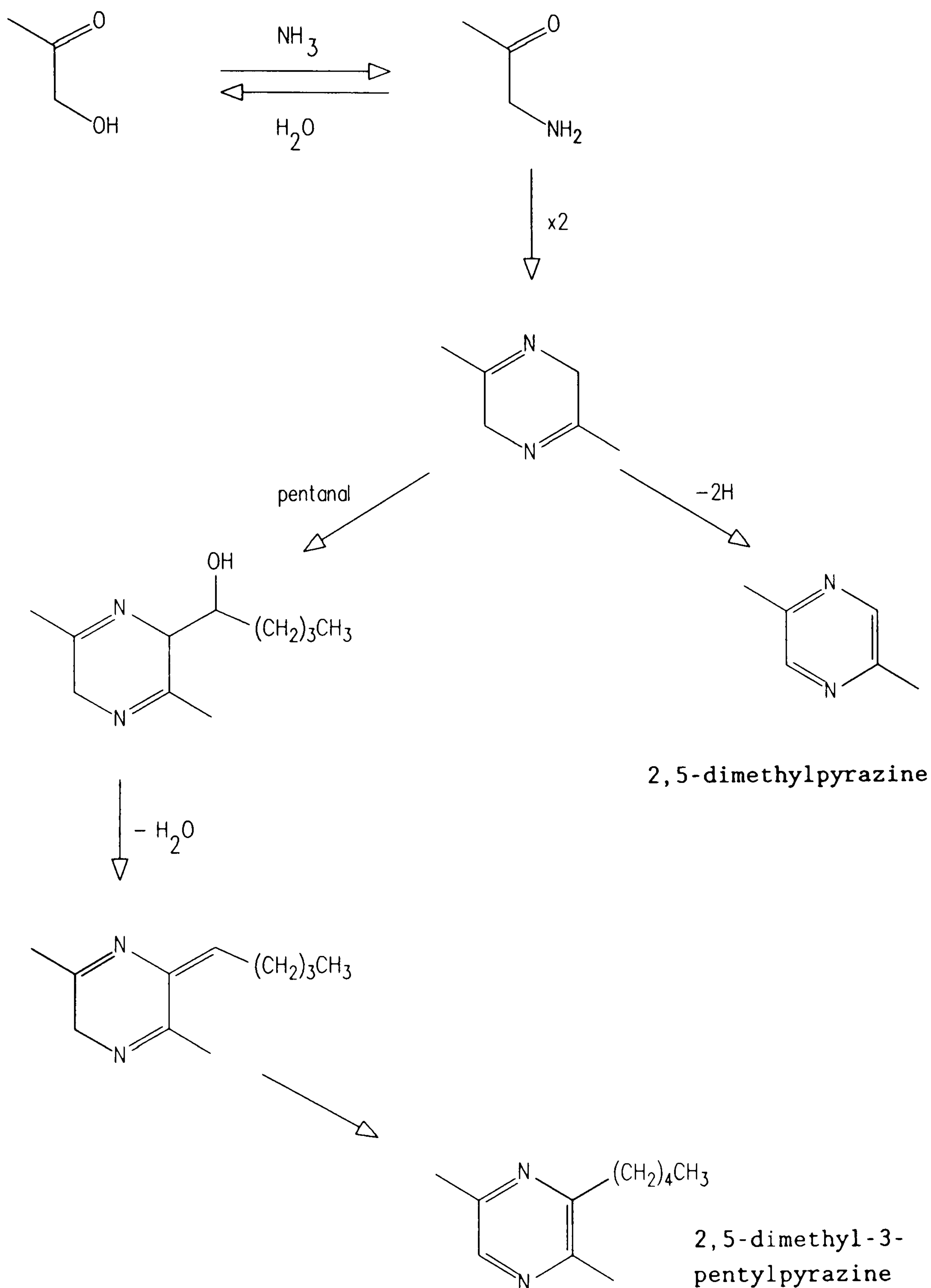
Products of the low temperature reaction between H_2S, NH_3 and several aldehydes (including butanal or 2-methylpropanal) include a range of dihydrodithiazanes and trithiolanes with propyl and isopropyl substituents (Kawai and Ishida 1987). Similarly, the inclusion of pentanal or hexanal in a reaction with acetaldehyde and ammonium sulphide gave rise to similar species with butyl and pentyl substituents (Hwang *et al* 1986).

The reaction of 1,4-dicarbonyl compounds with H_2S gives alkylthiophenes (Boelens *et al* 1975), while propenyl substituted dithiins have been obtained from the reaction of H_2S and 2-butenal (Badings *et al* 1976). A range of long-chain sulphur-containing heterocyclic compounds have been identified from the reaction between the lipid oxidation product, 2,4-decadienal, and cysteine or glutathione. These include thiophenes, a trithiolane, tetrathianes and dithiazanes, with substituents of five to seven carbons, originating from the aldehyde (Zhang and Ho 1989). It is suggested that 2,4-decadienal breakdown products, such as hexanal, are probably involved in the formation of these compounds.

1.3.4 CONTRIBUTION OF LIPID-MAILLARD INTERACTIONS TO THE FORMATION OF DESIRABLE FOOD FLAVOURS.

While the effect of oxidizing lipids on the degradation of proteins and the ability of Maillard products to inhibit the degradation reactions of lipids have both received considerable attention over the last twenty years, the role of lipid-Maillard

Figure 1.3A: Mechanism proposed for the formation of 2,5-dimethylpyrazine and 2,5-dimethyl-3-pentylpyrazine from acetol, ammonium acetate and pentanal (Chiu et al 1990).



interactions in the formation of desirable flavours in foods has been little studied.

Ho *et al* (1987) have identified a number of long-chain heterocyclic compounds indicative of interaction between lipid and the Maillard reaction in deep fried foods. Long-chain pyridines, pyrazines, thiazoles, oxazoles and trithiolanes have been identified in the volatiles from deep-fried chicken and potatoes (Tang *et al* 1983; Carlin *et al* 1986; Ho *et al* 1987). Some possess odours reminiscent of fried foods, suggesting that interactions between Maillard reactions in the meat and the frying fat may contribute to the flavour of these fried foods. The levels of these compounds in chicken and potatoes cooked without fat are not reported, so some of these compounds may arise by the reaction of Maillard reactants with lipids contained within these foods. Such a hypothesis is supported by the detection of a range of long-chain alkyl thiazoles in the headspace volatiles collected from various beef muscles, cooked without added fat (Farmer, unpublished results).

Ohnishi and Shibamoto (1984) looked at the contribution made to the flavour of cooked meat by the volatile compounds obtained from beef fat heated with glycine, but found that the flavour of the reaction mixture was unpleasant rather than pleasant or cooked.

Lipid may also affect the formation of flavour volatiles by acting as a solvent for reactions. The Maillard reaction between leucine or valine and fructose yields 3-methylbutanal and 3-methylpropanal, which are considered important indicators of quality during the processing of cocoa to make chocolate (Arnoldi *et al* 1987). The formation of these compounds occurs much faster in a mixture of cocoa butter and water than in water alone and, therefore, it is proposed that cocoa butter may contribute to the formation of the final flavour.

The role of lipid in the generation of the desirable flavour and aroma of cooked meat has been of particular interest at the Institute of Food Research-Bristol for a number of years. The volatile compounds from cooked lean beef or pork are dominated by aldehydes and alcohols, presumed to originate from the breakdown of unsaturated lipids (Mottram *et al* 1982). However, the addition of beef adipose tissue to give a nine-fold increase in lipid

content did not cause a proportional increase in aliphatic volatiles. Therefore, it was suggested that many of these volatile compounds may originate from the structural lipids rather than intramuscular triglycerides. Subsequent work assessed the role of triglycerides and structural phospholipids in the formation of meat aroma by examining meat from which either the triglycerides only, or the total lipids (including the phospholipids), had been extracted (Mottram and Edwards 1983). Removal of the triglycerides had little effect on the aroma of the cooked meat or the pattern of volatile compounds observed using GC-MS. However, the additional removal of phospholipids changed the odour description from "meaty" to "roasted, toasted" and also caused a marked alteration in the volatile products; the aliphatic products of lipid oxidation declined, while the quantities of pyrazines were greatly increased. Thus, it appears that phospholipids are indeed the primary source of the lipid degradation products which dominate cooked meat volatiles. In addition, some interaction occurs in meat between phospholipids and the Maillard reactants responsible for pyrazine formation, which may be responsible for the development of meat flavour.

More recently, MacLeod and Ames (1987) also compared the volatiles obtained on cooking reconstituted, freeze dried, defatted beef with those from the untreated meat. Although certain pyrazines were enhanced in the defatted meat, an overall increase in these compounds was not observed and the odour of the defatted meat was not described as roasted, perhaps due to differences in the treatment and final water content of the reconstituted beef. However, these authors did observe that the extraction of all lipid removed the meaty odour of the cooked meat and increased the quantities of some Maillard products, thus supporting the hypothesis that phospholipid-Maillard interactions contribute to the generation of meat aroma.

1.4 AIMS OF RESEARCH

The Maillard reaction between amino acids and reducing sugars is known to yield a wide variety of volatile products of importance to the flavour and aroma of many cooked foods (Sec. 1.1). Similarly, the reactions occurring during the oxidation of lipids give many aliphatic volatile products which are widespread in raw and cooked foods (Sec. 1.2). The headspace volatiles from cooked meats include many products from both these reactions. However, despite the identification of more than a thousand components of beef aroma (Mottram 1990), the role of individual compounds in the characteristic aroma of cooked meat is the subject of continuing research around the world.

As described in the previous Section, investigations into factors affecting the formation of meat aroma showed that, while some lipid was essential for the formation of meaty aroma, all the triglyceride could be removed with little effect on aroma or volatile products; sufficient lipid was supplied by the structural phospholipids for aroma formation (Mottram and Edwards 1983). The proposal that phospholipid may interact in the Maillard reaction led to the study of this phenomenon using model systems. Preliminary work (Whitfield *et al* 1988) established that the addition of phospholipid to a simple model Maillard system containing one amino acid and a reducing sugar did indeed affect the formation of volatile compounds. This Thesis describes the use of such model systems to study the nature of the reactions occurring between lipids and Maillard reactants.

Two questions arose from the studies on meat. It was evident that the removal of triglyceride from meat had little effect either on the volatile products or aroma, while the additional removal of phospholipids caused extensive changes to both. However, it was not clear whether phospholipid itself was a prerequisite of desirable aroma in meat or whether the presence of a small quantity of any lipid, eg triglyceride, would have the same effect. Furthermore, if phospholipid did prove to have a characteristic effect on the Maillard reaction, could model systems be used to deduce which constituent of phospholipid is responsible?

Before it was possible to undertake a detailed comparison of the extent of participation of different lipid classes in the Maillard reaction, it was necessary first to identify the major volatile components of phospholipid-containing Maillard systems. This part of the work is described in Chapter 2 for the Maillard reactions between glycine or cysteine and ribose, in the absence and presence of phospholipid.

Chapter 3 describes a comparison of the effect of beef triglyceride, beef phospholipid and two egg phospholipids on the Maillard reaction between cysteine and ribose. This study was designed to address the two issues mentioned above and also provide information on the effect of Maillard reactants on the formation of lipid oxidation products.

Phospholipids are major structural constituents of the cell walls of all living tissues, while amino acids and sugars are ubiquitous in the aqueous cell contents. Thus, it might be expected that reactions between these components would play an important part in the generation of flavour during the cooking of many foods. However, relatively little is known about the effect of lipid-Maillard interactions in the generation of desirable food flavour; in particular, interest in phospholipids has largely concentrated on their propensity for autoxidation and the production of off-flavours. The investigations described in this Thesis were designed to shed some light on the possible role of reactions between Maillard reactants and phospholipids in the formation of aroma compounds in cooked foods.

CHAPTER 2

IDENTIFICATION OF VOLATILE PRODUCTS OF THE REACTION OF GLYCINE OR CYSTEINE WITH RIBOSE, IN THE ABSENCE OR PRESENCE OF PHOSPHOLIPID

2. IDENTIFICATION OF THE VOLATILE PRODUCTS OF THE REACTION BETWEEN GLYCINE OR CYSTEINE AND RIBOSE, IN THE ABSENCE OR PRESENCE OF PHOSPHOLIPID

The effect of phospholipid on the products of the Maillard reaction was studied using two model systems containing one of the amino acids, glycine or cysteine and the reducing sugar, ribose; glycine was chosen because it is the simplest amino acid and cysteine due to its known importance in the formation of meat-like flavours (Morton *et al* 1960; Kiely *et al* 1960). It has been shown (Kiely *et al* 1960) that the identity of the reducing sugar has little influence on the aroma character of the products of the Maillard reaction but that it has a considerable effect upon the rate of reaction; thus, ribose has been used throughout these studies due to its high reactivity in the Maillard reaction (Pomeranz *et al* 1962; Lane and Nursten 1983), as well as its recognised role in the production of meat flavours (May 1974).

The Maillard reactions between either glycine or cysteine and ribose yield many hundreds of volatile products, predominantly cyclic compounds containing the heteroatoms, oxygen, nitrogen and sulphur. The addition of a lipid introduces many more compounds, including not only the aliphatic products of lipid degradation but also long chain heterocyclic compounds whose formation is specific to the interaction of lipid in the Maillard reaction.

A detailed assessment of the effect of different lipids on the generation of aroma compounds by these reactions had to await the identification of the major volatile products. Thus the following study was planned with two main objectives.

(a) To identify the major products of the Maillard reactions between glycine or cysteine and ribose in the absence and presence of phospholipid.

(b) To assess the impact of the added phospholipid both on the overall odour characteristics of the reaction products and on the individual aromas separated by capillary GC.

2.1 OUTLINE OF METHOD

A full description of the experimental details for this experiment can be found in Section 6; the following is a brief summary of the method:

Solutions of glycine or cysteine (5 mg ml^{-1}) and ribose (4.5 mg ml^{-1}) in phosphate buffer (pH 5.7, 0.2M) were heated at 140°C for 1 hour with and without the inclusion of an egg phospholipid (15 mg ml^{-1}). A solution of ribose (4.5 mg ml^{-1}) was treated similarly. The phospholipid comprised approx. 70% phosphatidylcholine and 30% phosphatidylethanolamine; it had a nitrogen content of 1.65% and a fatty acid composition: 16:0 (30%), 18:0 (15%), 18:1 (28%), 18:2 (15%), 20:4 (7%), others (5%). After cooling, the reaction mixtures were transferred to Erlenmeyer flasks fitted with Dreschel heads and the volatile compounds from each reaction mixture were swept on to Tenax traps with a stream of nitrogen. Gas chromatography was performed on a WCOT fused silica capillary column coated with CPWAX57CB (Chrompak Ltd). The trapped volatiles were desorbed directly on to the front of the column, a section of which was cooled with solid CO_2, prior to chromatography over the temperature range 60°C to 200°C

For aroma assessment the effluent was split between a flame ionization detector and an 'odour port'; the eluting odours were appraised by four individuals who recorded their descriptions and made synchronous marks on the GC recorder trace.

Mass spectral data were obtained on Finnigan 4000 and Kratos MS80RFA (high resolution) mass spectrometers operating in the GC-MS mode. Vapour phase infrared spectroscopy was performed using a Hewlett Packard HP5890 GC fitted with an HP5965A infrared detector.

2.2 VOLATILE AROMA COMPOUNDS PRODUCED IN MAILLARD REACTIONS INVOLVING GLYCINE, RIBOSE AND PHOSPHOLIPID: RESULTS AND DISCUSSION.

The major headspace components of the glycine + ribose and the glycine + ribose + phospholipid Maillard reaction systems are listed in Table 2.2a in order of elution; the 109 compounds presented are those which gave significant peaks (greater than 100,000 ions in height in the GC-MS chromatogram), together with any minor components of known odour significance. For each compound the linear retention index (LRI) is given, together with an indication of its relative concentration in each reaction mixture and the technique(s) used for identification. Wherever possible, authentic compounds were obtained and their mass spectra and LRI values used to confirm the identities of volatile components of the reaction mixture. Mass spectral data are included where a reference spectrum was not available. The accurate mass and the major absorptions in the vapour phase infrared spectra are also listed where these techniques aided identification. The compounds include hydrocarbons (7), alcohols (9), aldehydes (11), straight-chain ketones (16), cyclic ketones (4) and heterocyclic compounds (52). The identities of 68 of these have been established by comparison of LRI and mass spectral data with those of authentic substances, and probable identities have been suggested for a further 32 compounds; nine components remain unidentified. Heterocyclic compounds dominated the volatiles from the basic glycine + ribose reaction system, while the phospholipid-containing system also produced significant amounts of long chain alcohols and aldehydes.

Typical gas chromatograms of the volatile products of the two reaction mixtures are shown in Figures 2.2A and B. A collation of the assessors' aroma descriptions is marked on each chromatogram; these do not represent all the aromas detected but indicate positions where there was agreement on the presence and/or nature of the aroma. Enlarged versions of Figures 2.2A and B, together with examples of GC-MS ion chromatograms, may be found in Appendix II.

Some of the results described in this Section have been published (Salter *et al* 1988).

Table 2.2a: Volatile components obtained from the reaction between glycine and ribose in the absence and presence of phospholipid

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS data[b]
1	decane[c]	+[d]	++	1000	MS + LRI	
2	trans-1,3-nonadiene	-	++	1047	MS	
3	2,3-pentanedione	+	+	1055	MS + LRI	
4	hexanal	+	+++	1088	MS + LRI	
5	undecane[c]	+	+	1100	MS + LRI	
6	2-butylfuran	+	+	1119	MS + LRI	
7	2,3-hexanedione	++	+	1127	MS + LRI	
8	3,4-hexanedione	++	+	1135	MS + LRI	
9	4-methyl-3-penten-2-one	+	+++	1136	MS + LRI	
10	pentyl acetate	-	+	1167	MS	
11	heptanal	+	+++	1182	MS + LRI	
12	2-heptanone[c]	+	+++	1183	MS + LRI	
13	dodecane	-	++	1200	MS + LRI	
14	pyridine	+	-	1209	MS + LRI	
15	trimethyloxazole	+	+	1215	MS + LRI	
16	2-pentylfuran	+	+++	1220	MS + LRI	
17	6-methyl-2-heptanone	-	++	1228	MS	
18	2-methylpyridine	+	-	1242	MS + LRI	
19	3-octanone	-	++	1244	MS + LRI	
20	1-pentanol	+	++	1251	MS + LRI	
21	methylpyrazine	++	+	1271	MS + LRI	
22	octanal[c]	+	+++	1280	MS + LRI	
23	3-hydroxy-2-butanone	++	++	1286	MS + LRI	
24	1-octen-3-one	+	+++	1292	MS + LRI	
25	tridecane	-	++	1300	MS + LRI	
26	1-hydroxy-2-propanone	+	++	1304	MS + LRI	
27	2-hexylfuran	-	++	1312	MS + LRI	
28	an octanedione	-	+++	1312	MS	
29	trans-2-heptenal	-	+++	1317	MS + LRI	
30	4-methylpyridine	+	-	1320	MS + LRI	
31	2,5-dimethylpyrazine	+++	+++	1328	MS + LRI	
32	ethylpyrazine +	+++	+	1334	MS + LRI	
33	2,6-dimethylpyrazine				MS + LRI	
34	2,3-dimethylpyrazine	++	+	1352	MS + LRI	

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS data[b]
35	1-hexanol	-	++	1354	MS + LRI	
36	2-methyl-2-cyclopenten-1-one	+++	++	1367	MS	
37	nonanal[c]	+	+++	1386	MS + LRI	
38	2-ethyl-6-methylpyrazine	++	+	1386	MS + LRI	
39	2-ethyl-5-methylpyrazine	+++	+	1394	MS + LRI	
40	2-ethyl-3-methylpyrazine	+	+	1407	MS + LRI	
41	trimethylpyrazine	+++	++	1412	MS + LRI	
42	2-heptylfuran	-	+++	1417	MS + LRI	
43	_trans_-2-octenal	-	+++	1424	MS + LRI	
44	unknown MW 108	+++	+	1429		79, 108(75), 39(51), 80(25), 77(25) 40(23), 52(15), 51(12), 65(11),41(10)
45	a nonen-2-one	-	+++	1432	MS	
46	2-ethyl-3,6-dimethylpyrazine	++	+	1449	MS + LRI	Baltes and Bochmann (1987)
47	2-furfural	+++	+++	1455	MS + LRI	
48	unknown	-	+++	1456	-	41, 43(93), 55(61), 97(52), 71(50) 70(38), 69(28), 67(26), 56(25), 42(25); 79(24), 83(21), 98(24), 111(14) 123(11)
49	1-heptanol	-	+++	1460	MS + LRI	
50	2,5-diethylpyrazine	++	+	1461	MS + LRI	ten Noever de Brauw _et al_. (1980)
51	2-ethyl-3,5-dimethylpyrazine	+++	++	1466	MS + LRI	
52	unknown	-	+++	1469		107, 164(18), 77(12), 108(8)
53	3- or 4-methyl-2-furfural	+++	+	1480	ms	110, 109(64), 53(37), 39(16), 81(14), 51(13), 50(8), 52(7), 111(7), 54(6)
54	tetramethylpyrazine	++	+	1483	MS + LRI	ten Noever de Brauw _et al_. (1980)
55	benzofuran	+	++	1486	MS + LRI	
56	2,4-heptadienal	-	++	1489	MS + LRI	
57	a nonadienone	-	+++	1489	MS, AM	AM(M+) = 138.1062: $C_9H_{14}O$
58	2-ethyl-1-hexanol	++	++	1493	MS + LRI	
59	3,5-diethyl-2-methylpyrazine	+	+	1496	MS	ten Noever de Brauw _et al_. (1980)
60	2-acetylfuran	+++	+++	1498	MS + LRI	
61	3-nonen-2-one	-	+++	1510	MS + LRI	
62	benzaldehyde	+	++	1510	MS + LRI	
63	1-(2-furyl)-2-propanone	+++	++	1513	MS	Stoll _et al_. (1967)

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS data[b]
64	an octadien-2-one	-	++	1516	MS	Heller and Milne (1978); ten Noever de Brauw et al. (1980)
65	pyrazines MW 150	+++	+	1517	ms	149, 150(84), 42(33), 39(32), 135(29) 56(28), 54(27), 41(25), 53(25), 67(20); 122(19)(mixture)
66	2-octylfuran	-	+++	1520	MS + LRI	
67	2-nonenal	-	+++	1531	MS + LRI	
68	2(1-propenyl)pyrazine	++	+	1546	MS + LRI	ten Noever de Brauw et al. (1980)
69	3-or 4-methyl-2-furfural	++	+	1549	ms	110, 109(95), 53(58), 51(16), 39(11) 50(11), 81(10), 111(6), 52(6), 38(5)
70	1-pentadecene	-	++	1552	MS	
71	1-octanol	++	+++	1564	MS + LRI	
72	2-propionylfuran	+++	+++	1571	MS + LRI	
73	unknown	-	+++	1574	AM	94, 81(72), 107(60), 79(46), 77(38) 54(34), 55(34), 53(28), 91(25), 39(23); 68(22), 110(22), 121(12), 178(21) AM = 178.1357 : $C_{12}H_{18}O$
74	cyclopent-3-ene-1,2-dione	++	+	1578	ms	42, 96(100), 68(54), 54(49), 40(47) 39(36), 50(12), 38(11), 37(6), 51(6)
75	2-pentylpyridine	-	++	1583	MS + LRI	
76	2-undecanone	-	++	1591	MS + LRI	
77	2-acetylpyridine	++	++	1597	MS + LRI	
78	2-acetyl-3 or 4-methylfuran	++	++	1601	ms	109, 124(44), 43(27), 53)(26), 39(16) 41(14), 97(13), 81(7), 110,(7), 69(5)
79	a dimethylfurfural	+++	+++	1607	ms, AM, ir	123, 124(95), 39(42), 67(29), 41(25) 65(18), 40(10), 125(9), 95(8), 51(7) AM(M+) = 124.0532 : $C_7H_8O_2$ 2940cm^{-1}(w), 2819(w), 2737(w), 1699(s), 1603(m), 1535(m), 1387(w), 1266(w), 781(m)
80	trans-2-octen-1-ol	-	+++	1613	MS + LRI	
81	cis-2-octen-1-ol	-	+++	1616	MS	
82	2-formyl-1-methylpyrrole	+++	++	1618	MS + LRI	
83	3,5-dimethyl-2-cyclohexen-1-one	-	+++	1624	MS + LRI	

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS data[b]
84	a pyrrole MW 137	+++	++	1629	ms	43, 137(81), 39(79), 94(61), 95(51) 67(35), 109(32), 66(22), 38(15), 41(13); 122(9)
85	1-(2-furyl)-3-butanone	+++	+++	1639	MS, AM	Stoll et al. (1967) AM(M+) = 138.0716 : $C_8H_{10}O_2$
86	unknown	-	+++	1641	AM	79, 108(64), 91(54), 120 (37), 77(36) 41(34), 55(30), 81(23), 53(22), 93(21); 133(9), 147(3), 176(4) AM(M+) = 176.1184 : $C_{12}H_{16}O$
87	unknown MW 192	-	++	1646	AM	82, 43(40), 121(34), 192(17), 95(16) 108(15), 54(14), 83(8), 110(7), 68(6) AM (M+) = 192.1510 : $C_{13}H_{20}O$
88	2-acetyl-1-methylpyrrole	+++	++	1654	MS + LRI	
89	1-nonanol	++	+++	1665	MS + LRI	
90	a pyrrole MW 151	+++	++	1665	ms	151, 43(79), 108(65), 53(62), 109(46) 80(42), 123(34), 81(27), 52(21), 39(13)
91	2-furanmethanol	++	++	1665	MS + LRI	
92	an ethylmethylfurfural	+++	+++	1681	ms, AM, ir	138, 123(74), 137(25), 41(24), 81(20) 39(19), 79(17), 109(17), 67(16), 53(16); 95(14) AM(M+) = 138.0653 : $C_8H_{10}O_2$ 2984 cm^{-1}(m), 2945(w), 2818(w), 2733(w), 1699(s), 1601(m), 1532(s), 1389(w), 1334(w), 777(m)
93,94	furans MW 124, 152	+++	++	1690	ms	124, 137(84), 123(83), 67(70), 41(62) 39(53), 152(50), 43(49), 95(41), 53(35); 109(18), 138(24)(mixture)
95	a pyrrole MW 151	++	+	1697	ms	151, 43(62), 53(57), 109(54), 108(51) 80(42), 52(16), 39(16), 51(11), 81(11)
96	unknown	-	+++	1705		67, 41(74), 43(67), 95(67), 96(52) 55(43), 81(40), 82(37), 57(37), 68(28); 109(21), 110(20), 124(27), 137(10) (mixture)
97	1-(5-methyl-2-furyl)-3-butanone	++	++	1712	MS	Stoll et al. (1967); Heller and Milne (1978)

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS data[b]
98	a pyrrole MW 165	++	++	1720	ms	165, 122(58), 164(41), 43(36), 123(25) 94(17), 95(15), 166(12), 67(10), 137(9)
99	naphthalene	+	++	1727	MS	
100	trans-2-undecenal	+	+++	1750	MS + LRI	ten Noever de Brauw et al. (1980)
101	a cyclohexenone MW 138	-	+++	1755	ms	82, 54(16), 39(14), 138(12), 41(9) 55(8), 53(5), 43(5), 83(5), 81(3)
102	a pyrrole MW 151	++	++	1760	ms	151, 108(75), 43(68), 53(62), 80(40) 109(38), 39(19), 123(17), 54(17), 81(15); 136(14)
103	trimethylfurfural	+++	+++	1766	ms, AM, ir	138, 137(92), 43(37), 81(26), 41(25) 39(21), 53(19), 79(18), 109(17), 67(11); 95(6), 122(6) AM(M+) = 138.0724 : $C_8H_{10}O_2$ 2935cm^{-1}(w), 2820(w), 2746(w), 1696(s), 1549(m), 1432(w), 1383(w), 1270(w), 1142(w), 820(w)
104	1-decanol	+	+++	1770	MS + LRI	
105	1-(2-furyl)-propane-1,2-dione	++	++	1772	MS	Stoll et al. (1967)
106	unknown MW 152	++	++	1806		137, 152(98), 41(39), 123(35), 109(24) 55(23), 79(22), 67(21), 95(21), 39(18)
107	2-tridecanone	-	++	1810	MS + LRI	
108	trans, trans-2,4-decadienal	-	+++	1815	MS + LRI	
109	1-(2-furfuryl)-pyrrole	++	++	1824	MS + LRI	

a. Methods of identification:

MS + LRI	Mass spectrum and LRI agree with these of authentic compound.
MS	Mass spectrum agrees with literature spectrum (reference given under MS/IR data).
ms	Interpretation of mass spectrum and comparison with those of related compounds.
ir	Interpretation of infrared spectrum and comparison with those of related compounds.
AM	Accurate mass data obtained by high resolution m.s. (details given under MS data).

b. Mass spectra are given where not previously reported in the literature. The ten most abundant ions are cited in order of decreasing relative intensity (excluding ions <2% base peak) followed by any other ions aiding interpretation. The molecular ion is underlined. For the other mass spectra the literature reference is Heller and Milne (1978) unless otherwise stated.

c. The presence of trace amounts of these five compounds in the reaction systems containing only cysteine and ribose may be due to cross-contamination from lipid-containing samples, although great care was taken in the cleaning and drying (150°C) of glassware and conditioning of traps (at least 1 hour at 250°C). The amounts found were extremely small compared with the quantities in the lipid-containing systems.

d. Relative size of peaks in GC-MS chromatogram: +++ large; ++ medium; + small; - not detected.

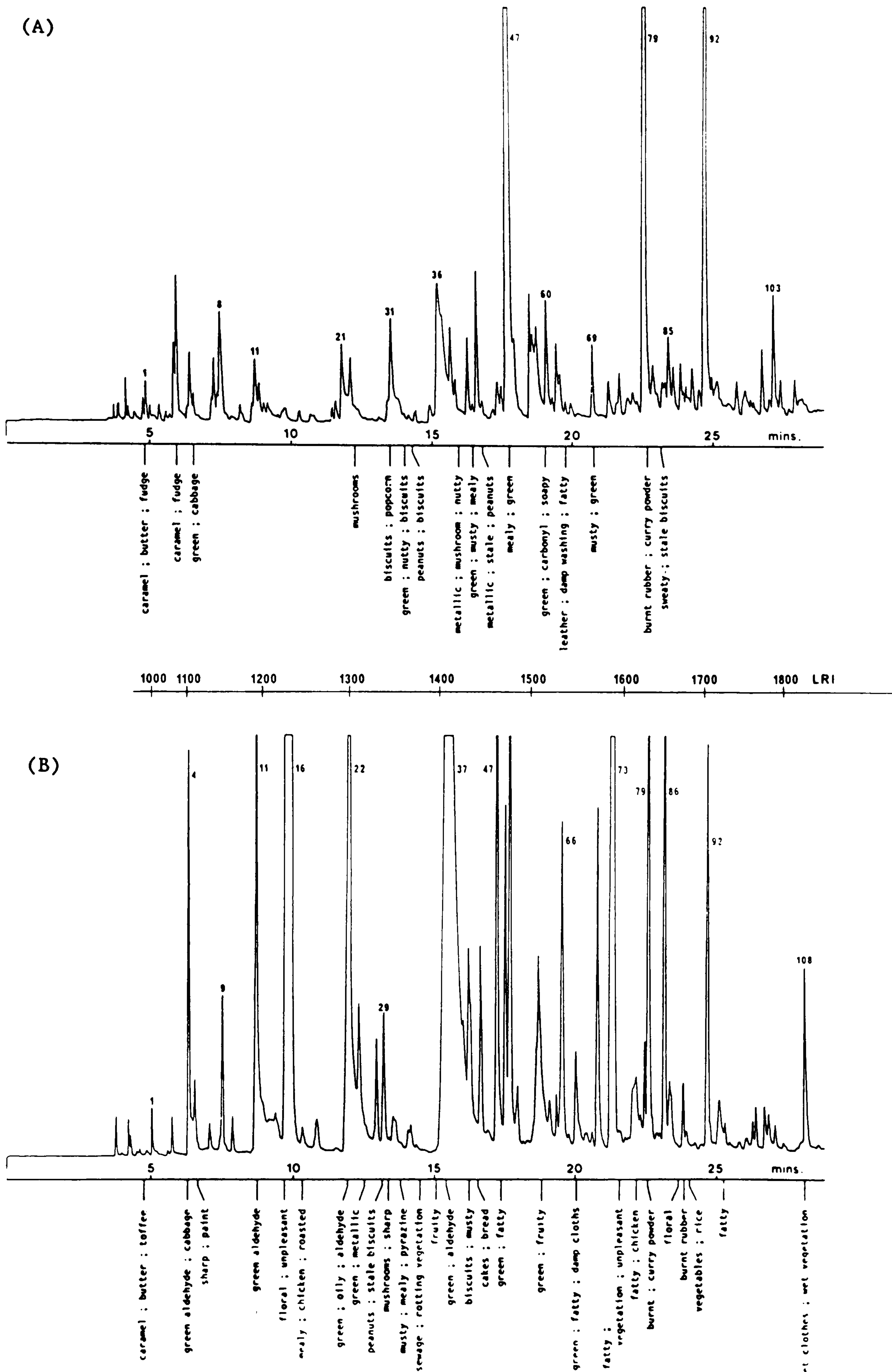


Figure 2.2A and B: Typical gas chromatograms of the volatile products of (A) The reaction between glycine and ribose and (B) the reaction between glycine, ribose and phospholipid, showing the positions of selected compounds (numbers refer to Table 2.2a) and a summary of the aromas detected in the column effluent. FID sensitivity: (A) 64 pA full scale, (B) 256 pA full scale.

2.2.1 IDENTIFICATION OF VOLATILE COMPOUNDS

A comparison of the two gas chromatograms shows that both the number and overall yield of volatile compounds evolved was much increased by the incorporation of phospholipid into the Maillard system. The major peaks in the glycine + ribose chromatogram were all heterocyclic compounds, and were dominated by 2-acylfurans. These compounds were still major components of the phospholipid-containing system but were accompanied by many compounds originating from the breakdown of the lipid, including hydrocarbons, alcohols, aldehydes, straight-chain and cyclic ketones. The formation of such compounds by lipid autoxidation has been discussed in Section 1.2.

Many of the volatile compounds collected from the glycine + ribose reaction mixture are characteristic products of the Maillard reaction. The identification and origin of these compounds will be discussed under each compound class.

Alkylfurans

Compound	MW	LRI	No.
2-Butylfuran	124	1119	6
2-Pentylfuran	138	1220	16
2-Hexylfuran	152	1312	27
2-Heptylfuran	166	1417	42
2-Octylfuran	182	1520	66

Most of the furans fell into two categories: the first comprised the 2-alkylfurans, which were found in large amounts in the phospholipid-containing mixtures and can be derived from fatty acids by autoxidation (Chang *et al* 1966: Nonaka *et al* 1967). The effect of Maillard reactants on the formation of alkylfurans will be discussed fully in Section 3.3.

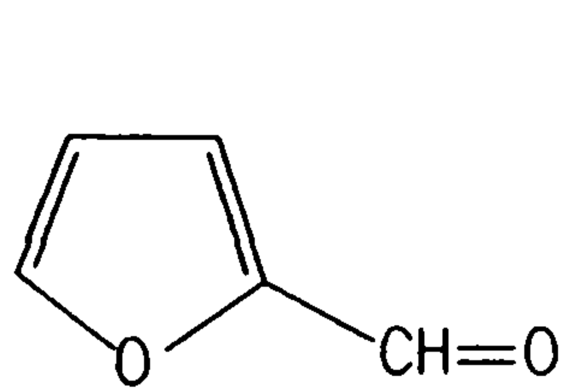
2-Acylfurans and furylketones

Compound	MW	LRI	No.
2-Furfural	96	1455	47
3 or 4-Methylfurfural	110	1480	53
3 or 4-Methylfurfural	110	1549	69
A dimethylfurfural	124	1607	79
An ethylmethylfurfural	138	1681	92
A trimethylfurfural	138	1766	103
2-Acetylfuran	110	1498	60
2-Acetyl-3 or 4-methylfuran	124	1601	78
2-Propionylfuran	124	1571	72
1-(2-Furyl)-2-propanone	124	1513	63
1-(2-Furyl)-3-butanone	138	1639	85
1-(5-Methyl-2-furyl)-3-butanone	152	1712	97
1-(2-Furyl)-1,2-propanedione	138	1772	105
Unknown furans	124/152	1690	93/94

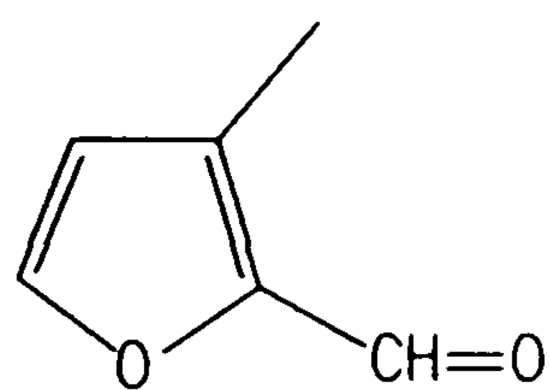
The second group of furans included 2-acylfurans and furylketones (Fig. 2.2C); these comprised many of the major components of the glycine + ribose headspace volatiles and possessed molecular weights: 96, 110, 124, 138 etc. A number of minor components also fell into this category. Some compounds were immediately identifiable by comparison with authentic samples. For others a tentative identification was possible only after careful comparison of the mass and infrared spectra with those of related compounds, and interpretation of accurate mass data from high resolution mass spectrometry. The mass and infrared spectra for these compounds are shown in Figures 2.2D and E.

One group of compounds warrants particular comment due to their predominance in the glycine-ribose gas chromatogram (peaks labelled **47**, **79**, **92** and **103** in Fig. 2.2A) and the fact that many of them have not been reported previously as components of model Maillard systems. Compound **47** was 2-furfural. High resolution MS of compounds **79**, **92** and **103** showed them to have the same general molecular formula, $C_nH_{2n-6}O_2$. Two methylfurfurals (compounds 53 and 69; MW = 110), a dimethylfurfural (79; MW = 124) and trimethylfurfural (103; MW = 138) all showed mass spectra strongly analogous to that of 2-furfural itself. Each possessed strong M^+

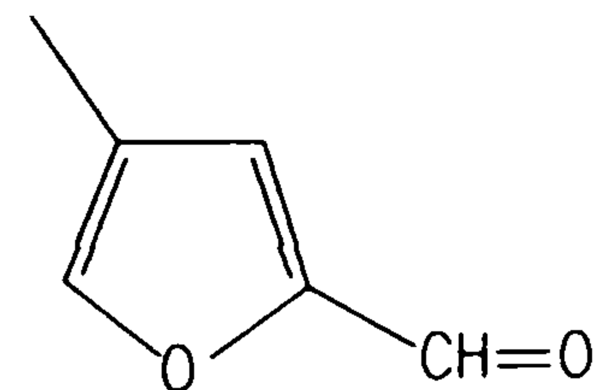
Figure 2.2C: Suggested identities of some 2-acylfurans and furylketones detected as volatile products of the Maillard reaction between glycine and ribose



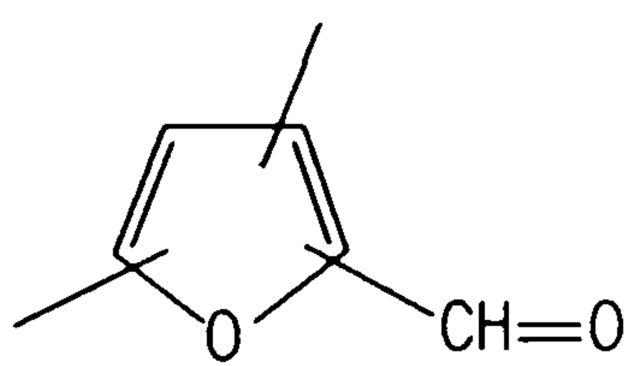
2-furfural (47)



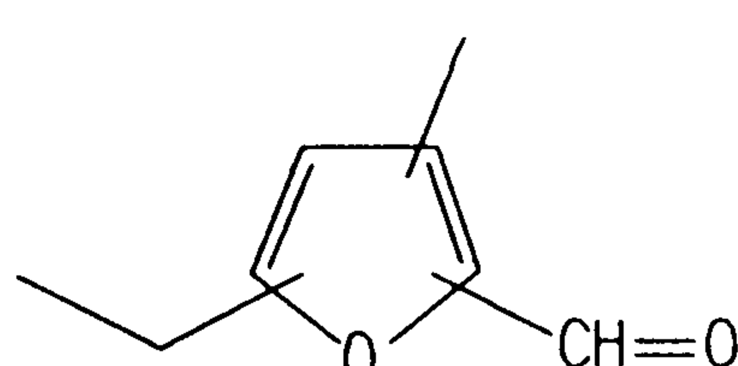
3-methyl-2-furfural (53/69)



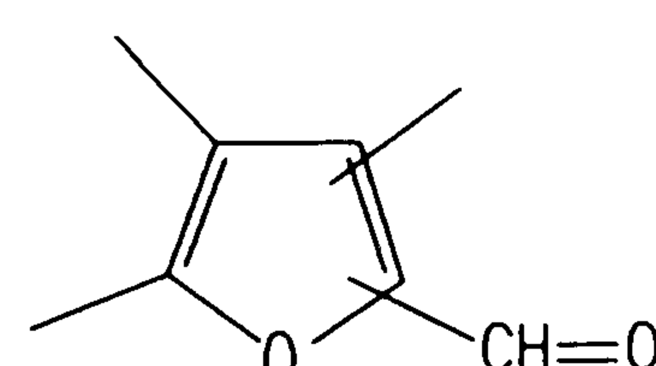
4-methyl-2-furfural (53/69)



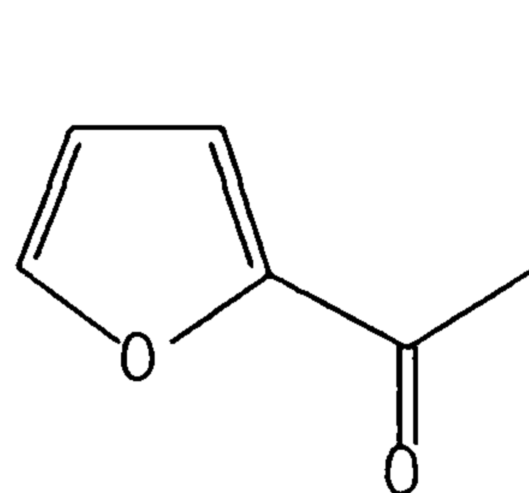
a dimethylfurfural (79)



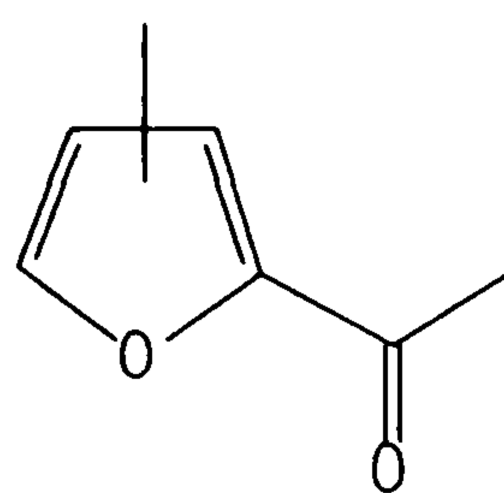
an ethylmethylfurfural (92)



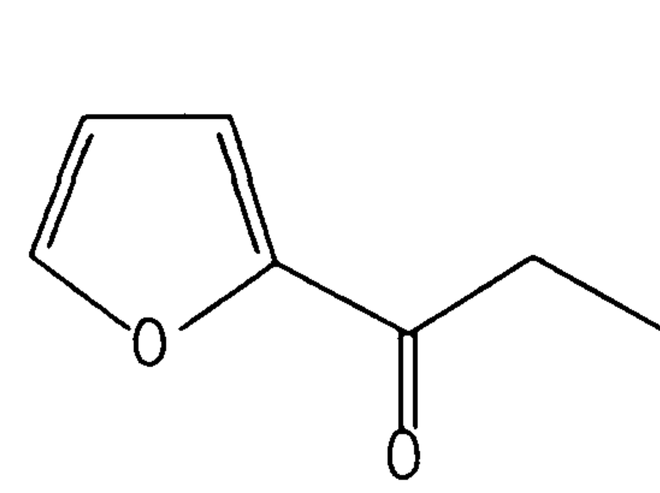
a trimethylfurfural (103)



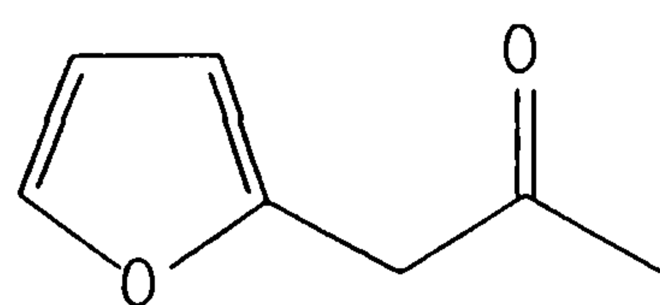
2-acetylfuran (60)



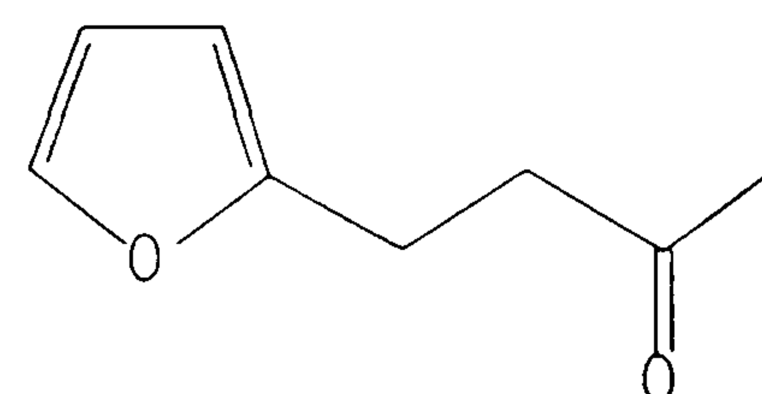
2-acetyl-3 or 4-methylfuran (78)



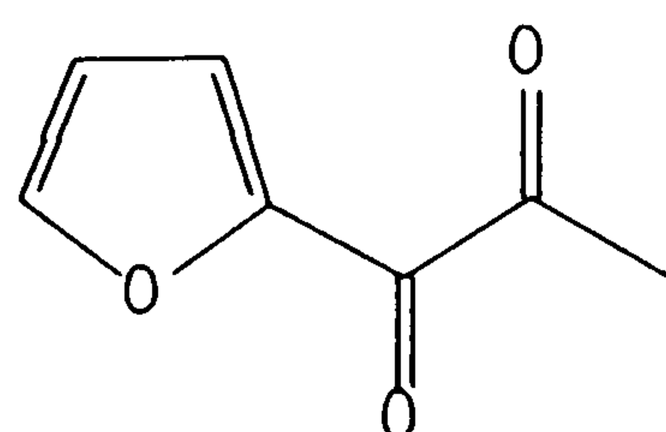
2-propionylfuran (72)



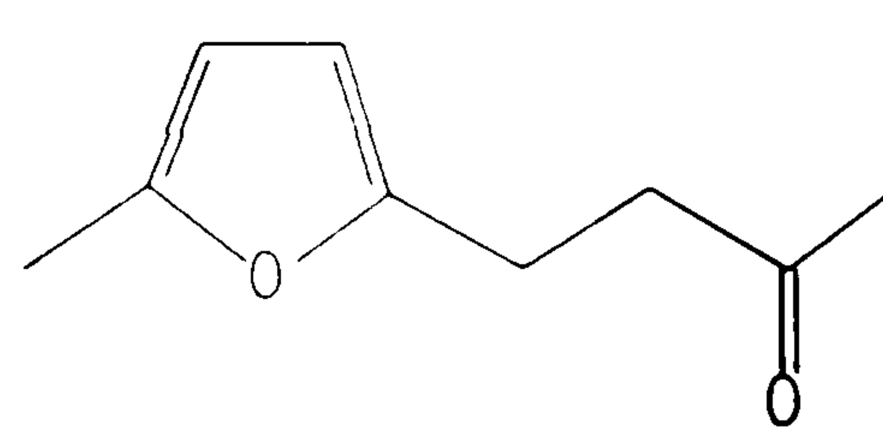
1-(2-furyl)-2-propanone (63)



1-(2-furyl)-3-butanone (85)



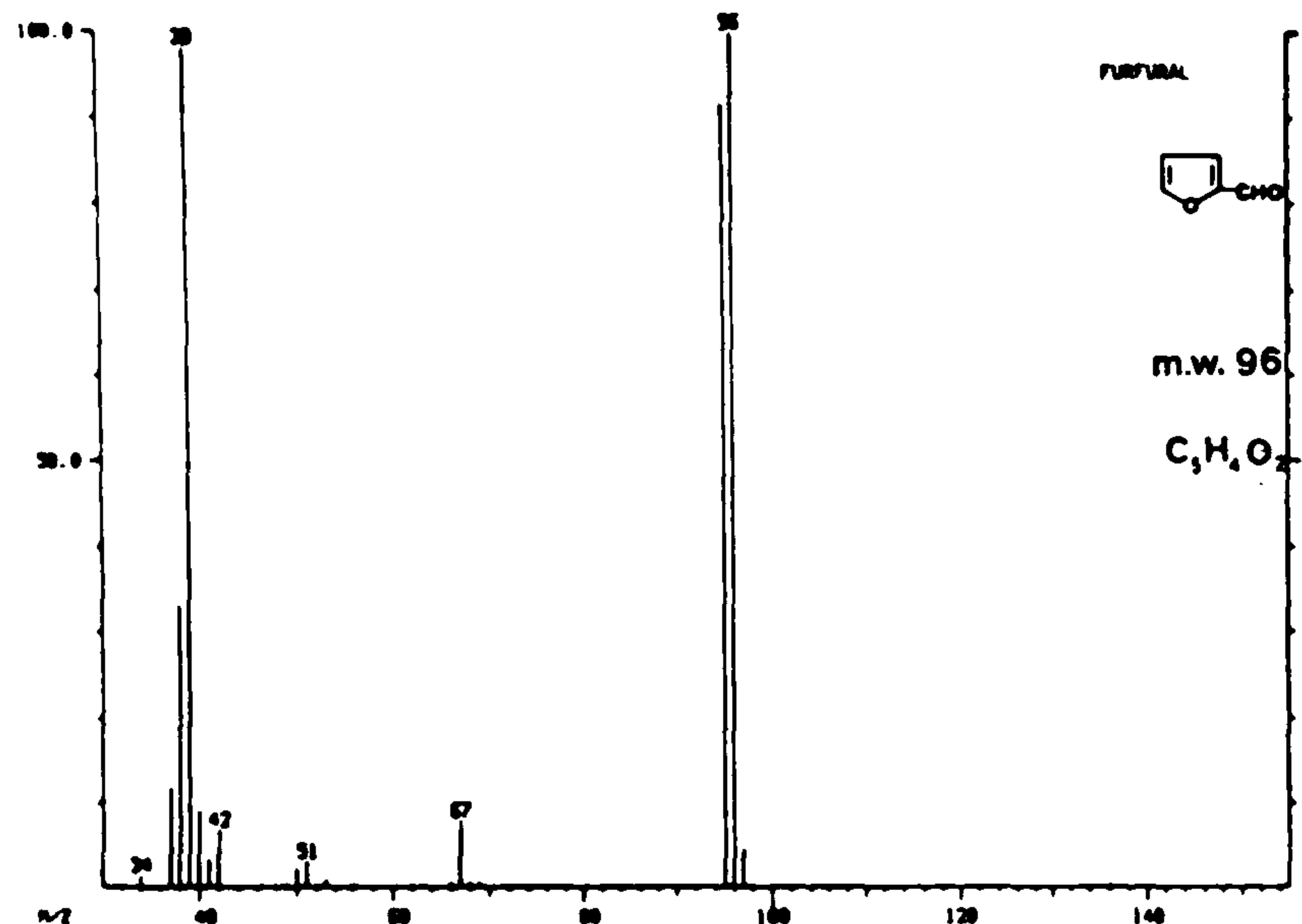
1-(2-furyl)-1,2-propanedione (105)



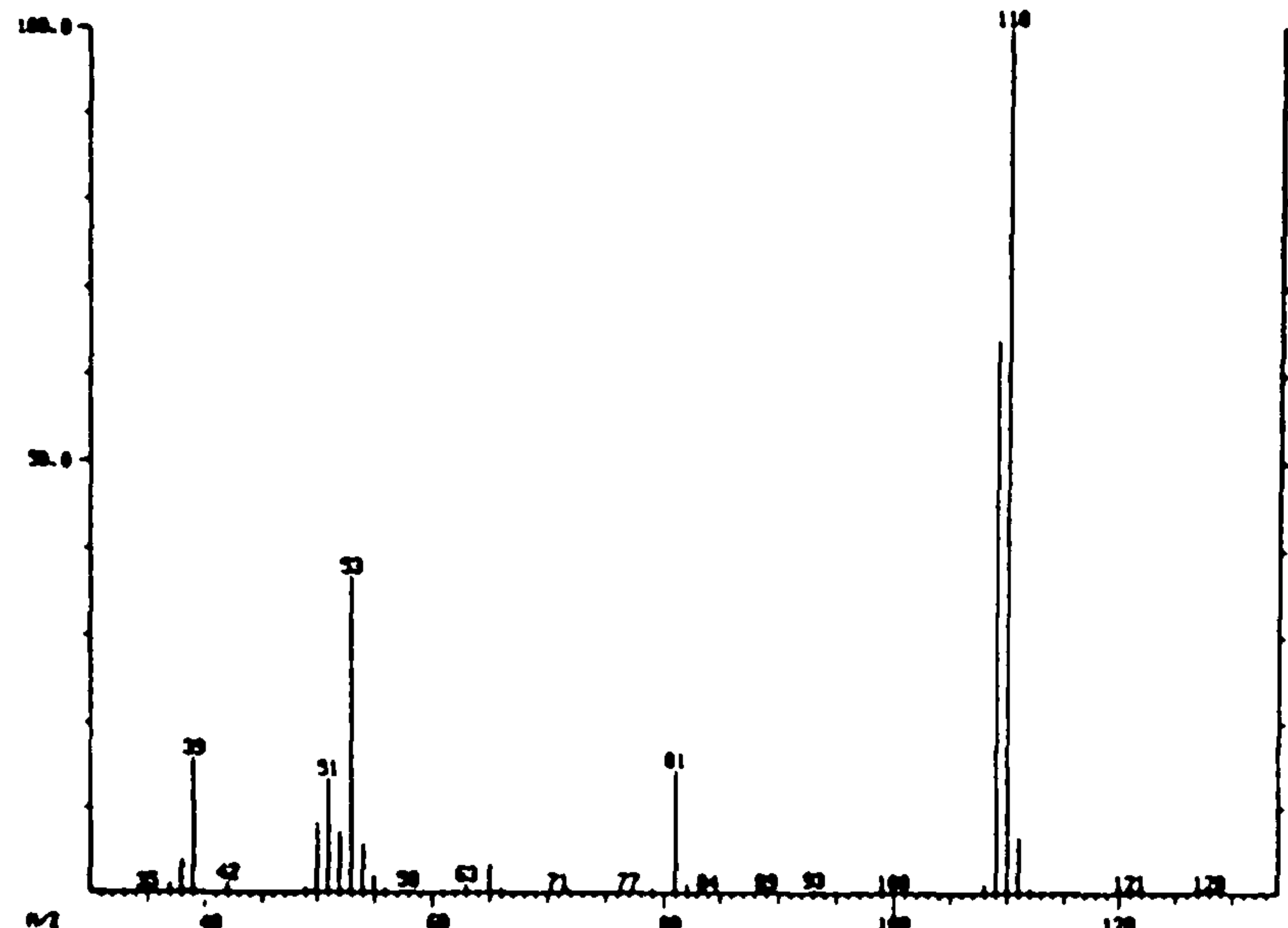
1-(5-methyl-2-furyl)-3-butanone (97)

Figure 2.2D: Mass spectra of furfurals from the reaction between glycine and ribose

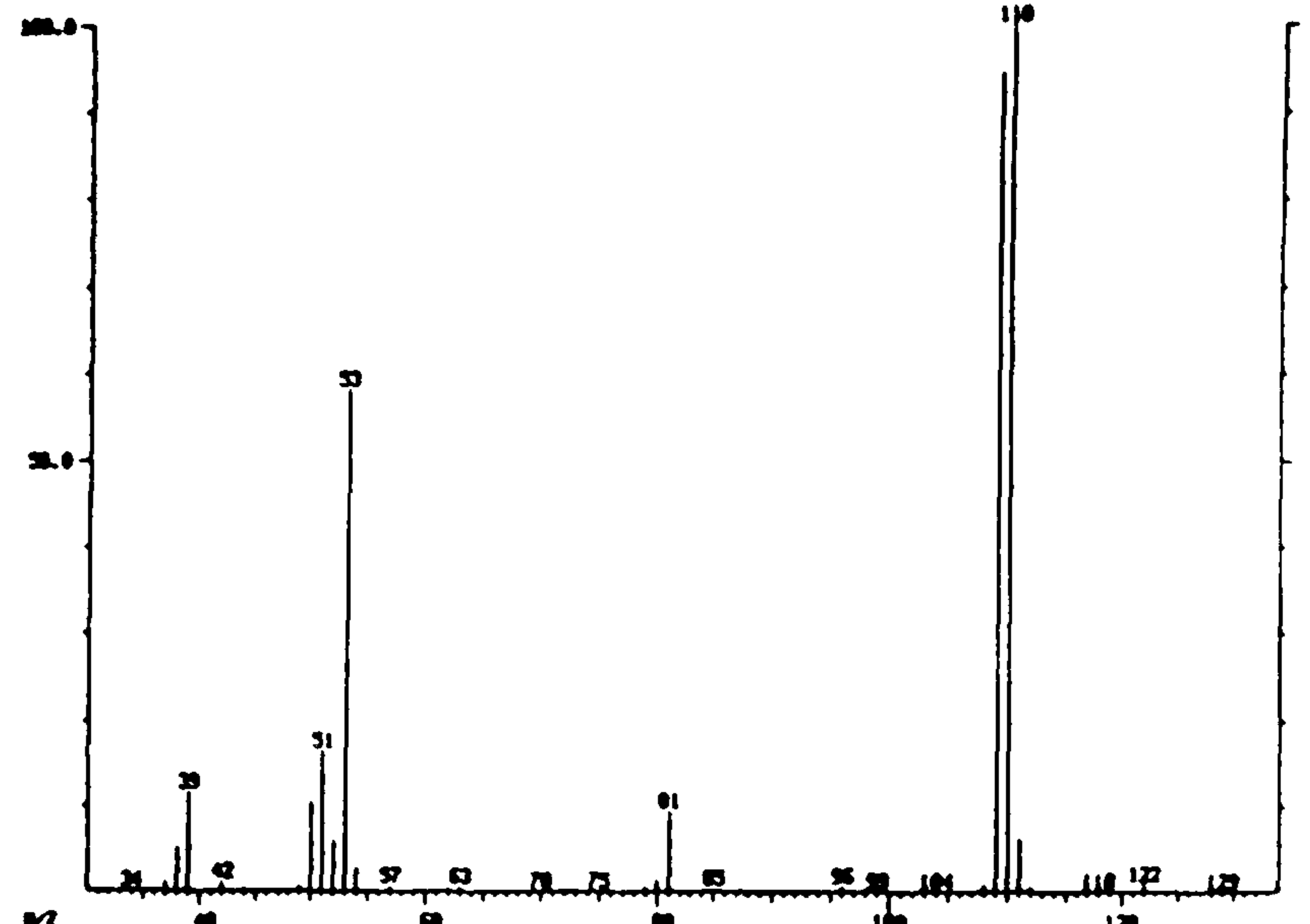
2-FURFURAL (compound 47)



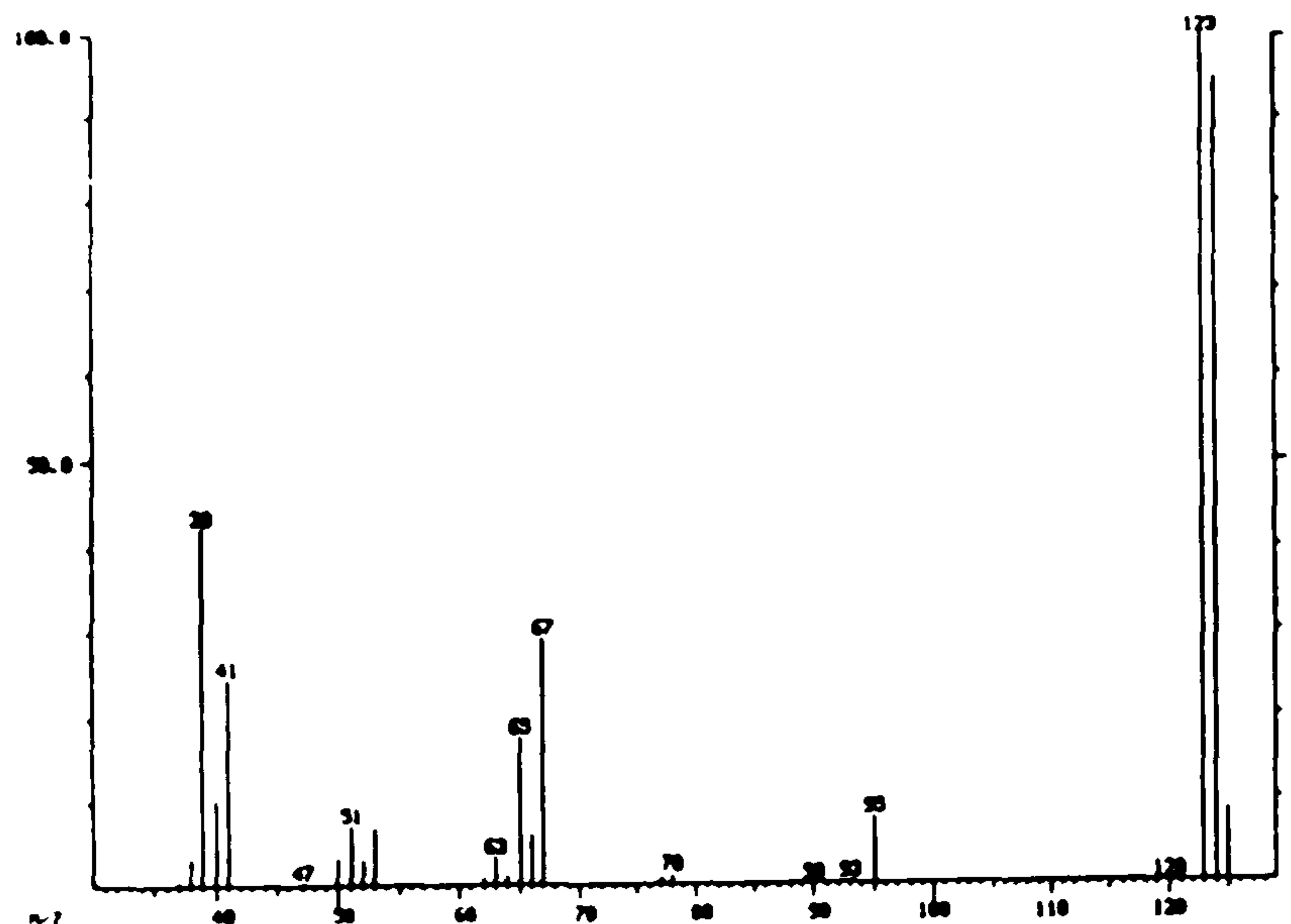
Compound 53 (3 or 4-methylfurfural)



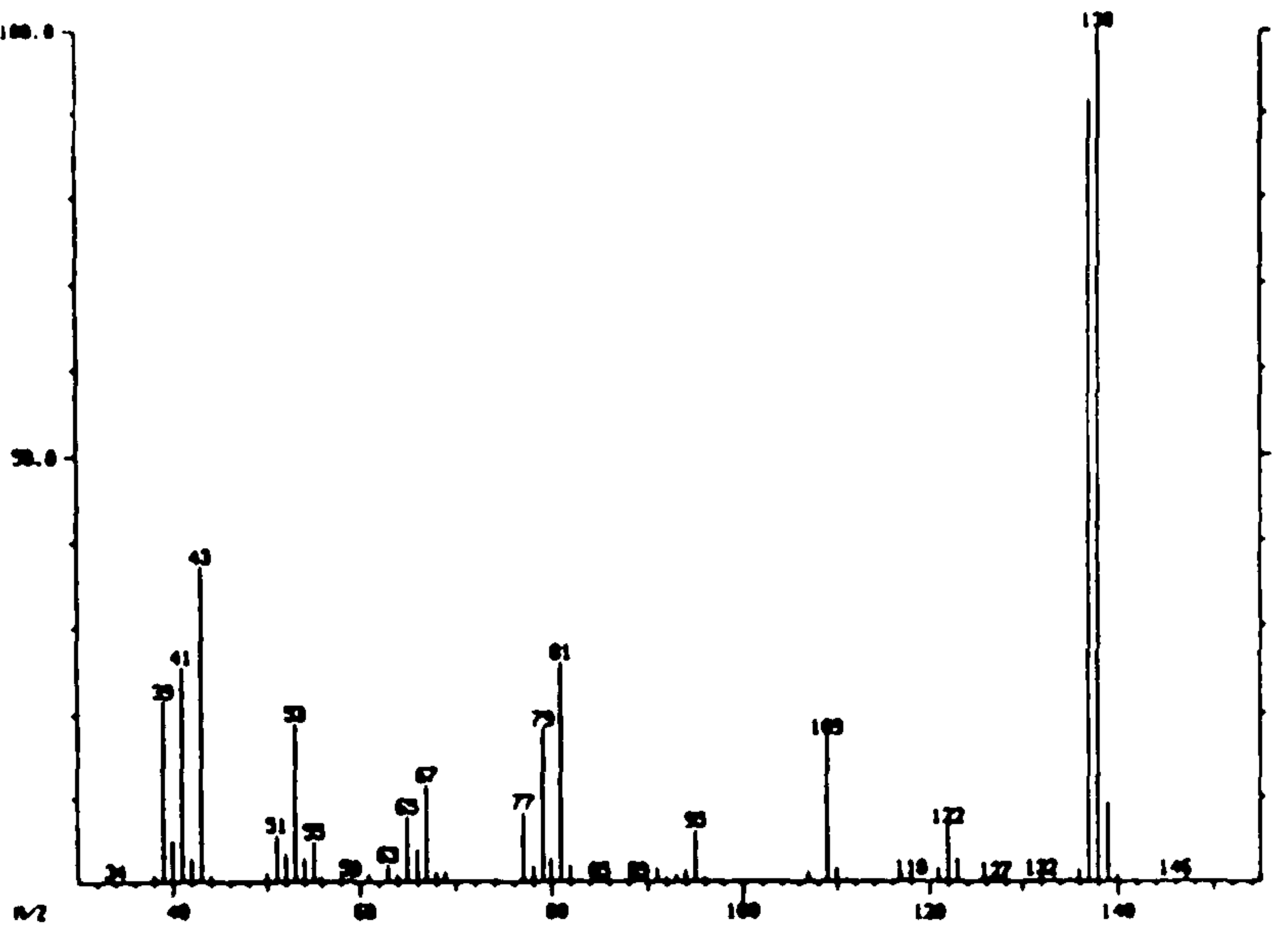
Compound 69 (3 or 4-methylfurfural)



Compound 79 (a dimethylfurfural)



Compound 103 (a trimethylfurfural)



Compound 92 (an ethylmethylfurfural)

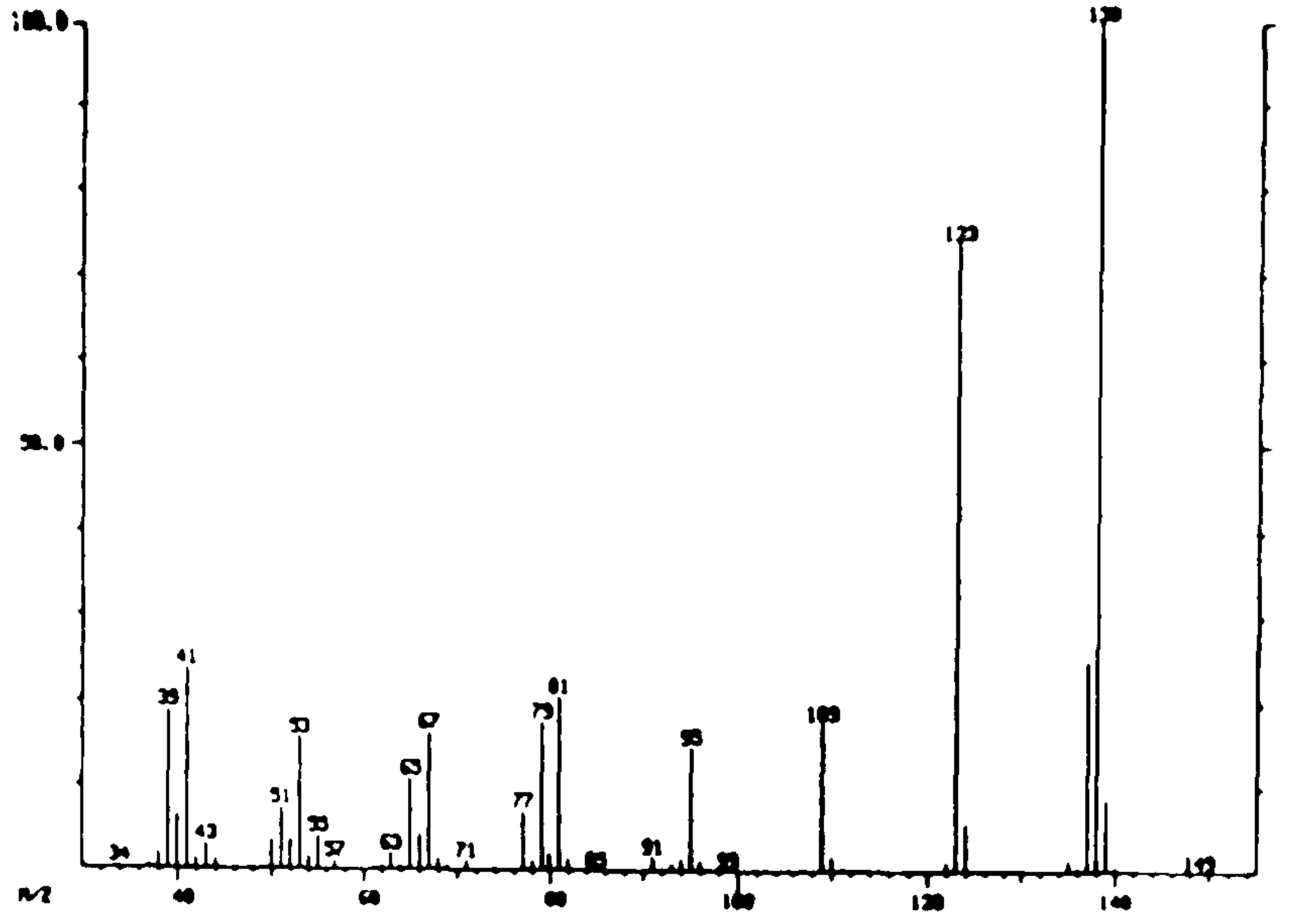
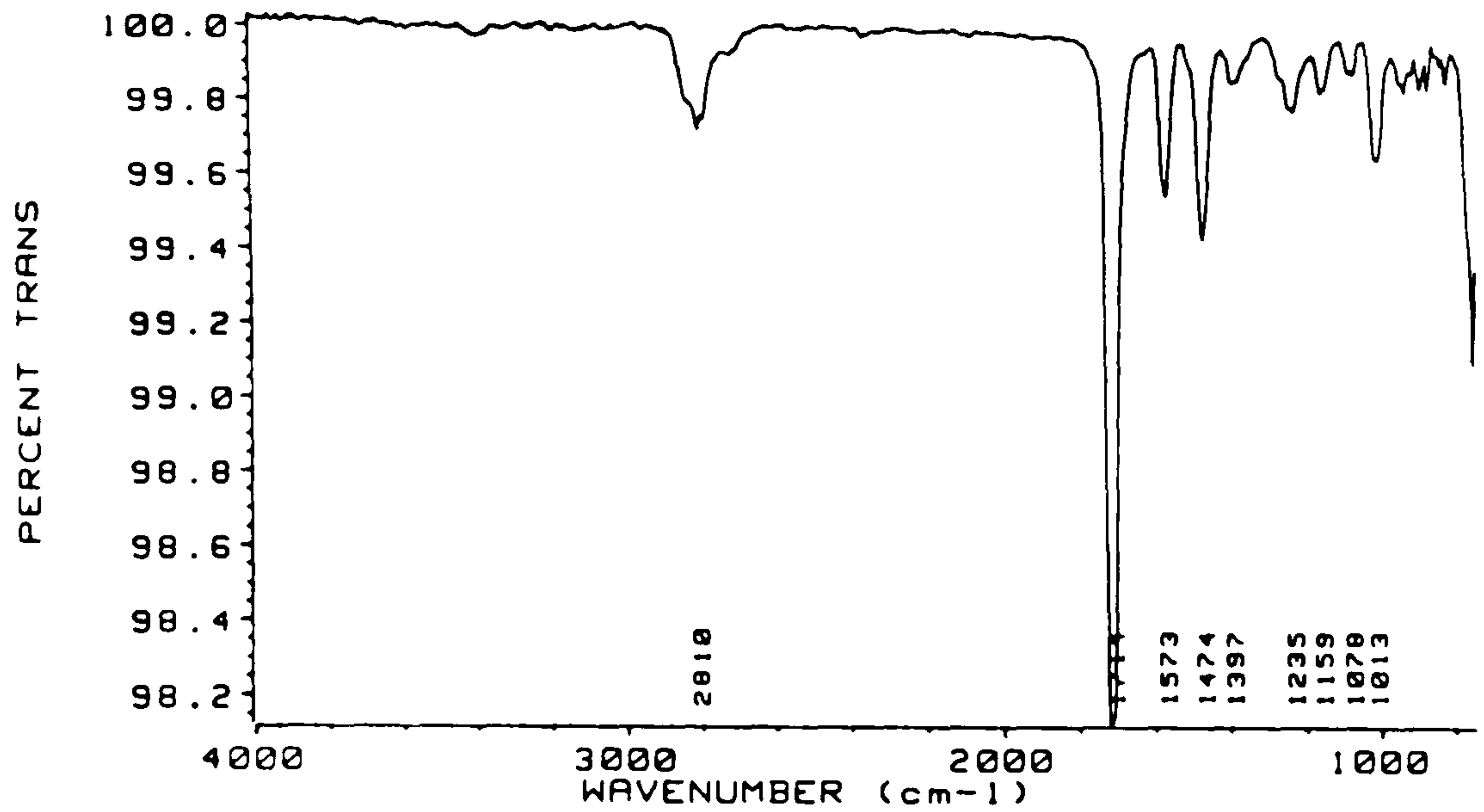
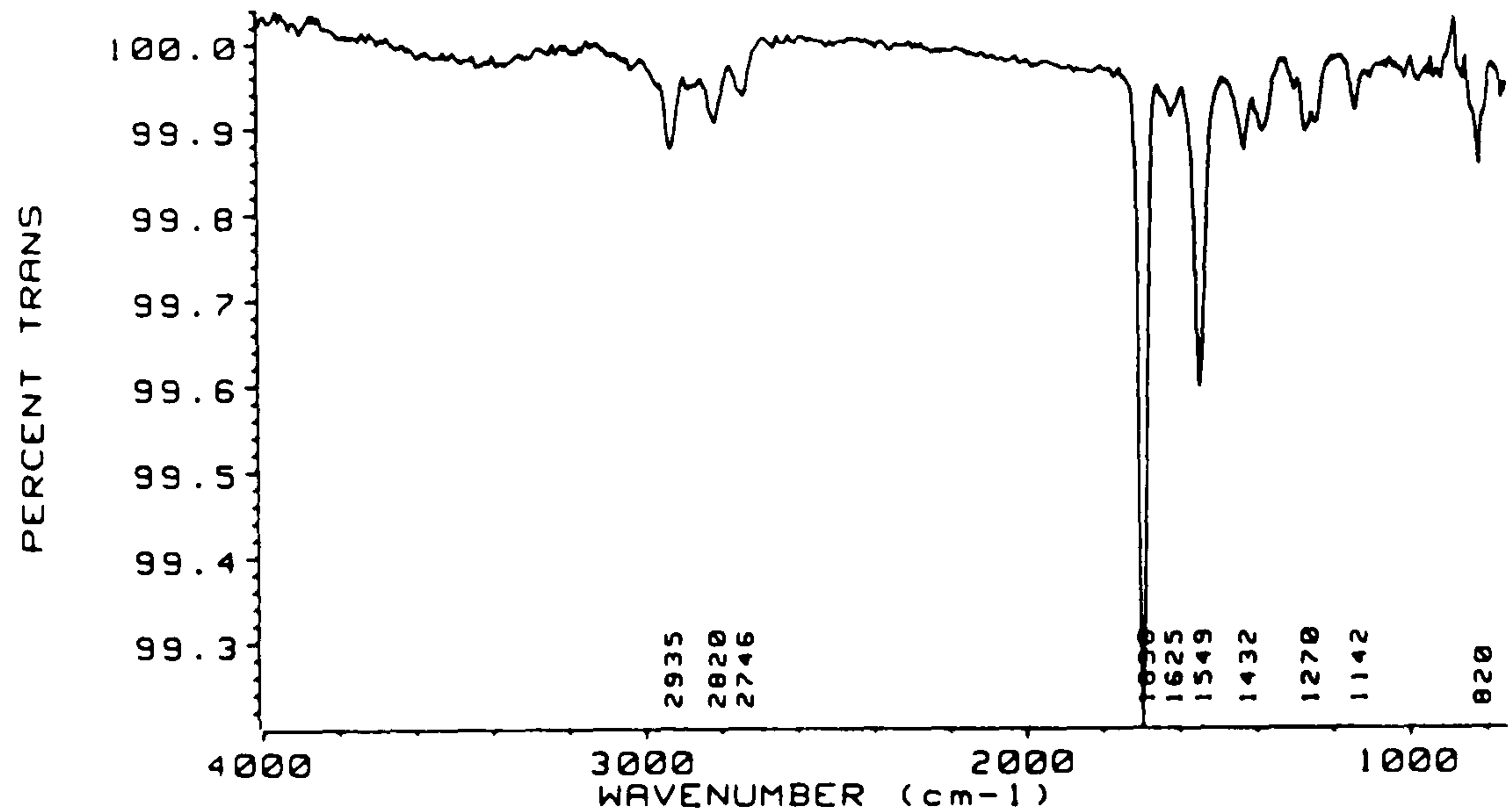


Figure 2.2E: Infrared spectra of furfurals from the reaction between glycine and ribose

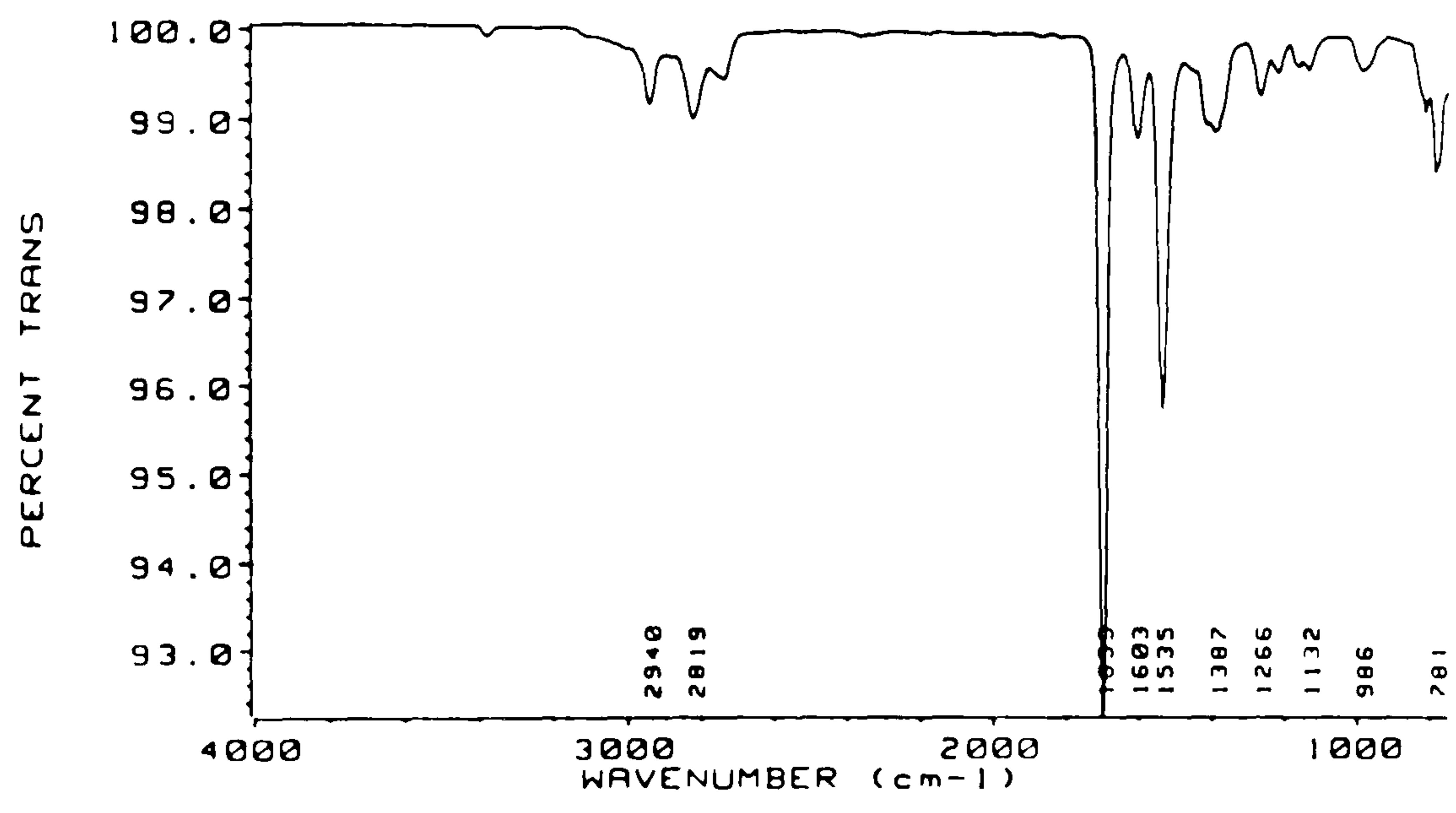
2-FURFURAL (compound 47)



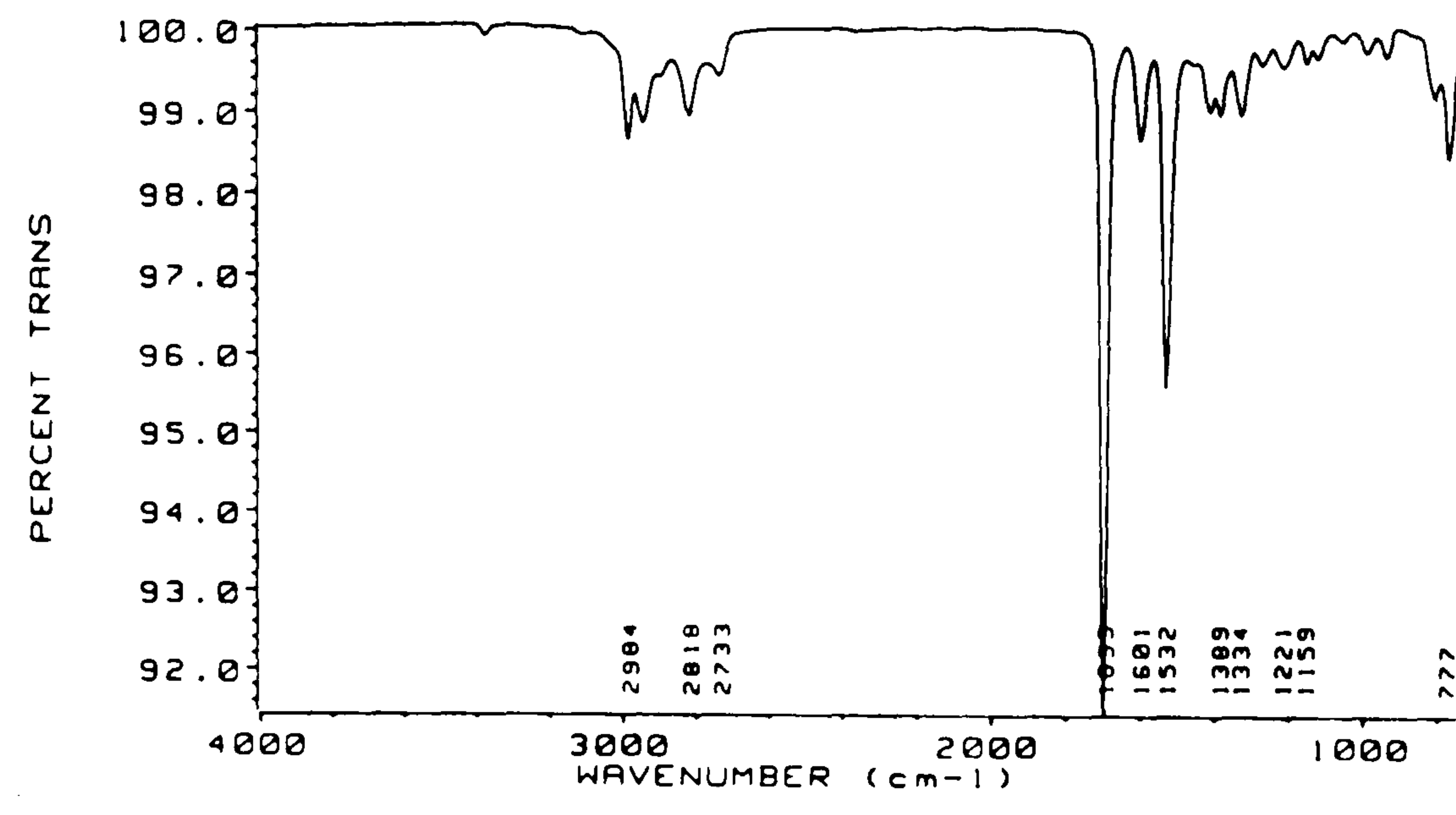
Compound 103 (a trimethylfurfural)



Compound 79 (a dimethylfurfural)



Compound 92 (an ethylmethylfurfural)



and M-1 ions (from the loss of the carbonyl H) with other major ions due to consecutive losses of two molecules of CO (Fig. 2.2F). The two methylfurfurals (LRI = 1480,1549) both had mass spectra very similar to that of 5-methyl-2-furfural (LRI = 1570) but since they eluted somewhat earlier, were deduced to be the 3- and 4-methyl-2-furfurals. The mass spectrum of compound 92 is analogous to that from an authentic sample of 5-ethyl-2-furfural (LRI = 1644), with the M-1 ion much smaller than that given by furfurals carrying only methyl substituents; the largest fragment arises from the loss of CH_3. A study of the accurate masses of the fragments suggested a tentative identity as an ethylmethylfurfural.

It is probable that the unknown furfurals 53, 69, 79 and 103 are 2-furfurals rather than 3-furfurals; their IR spectra are closely related to those of authentic 2-furfurals and there is no evidence for the presence of a second series of 3-furfurals. However, the possibility of 3-substitution cannot be ruled out as analogous 3-formylthiophenes have been detected (Section 2.3). It is hoped, in the future, to collect sufficient of these compounds to establish their substitution pattern by NMR. In the meantime, it is tentatively suggested that these compounds are 2-furfurals and the following speculations on the positions of alkyl substitution are based on this assumption.

The infrared spectra of the proposed dimethyl-, ethylmethyl- and trimethylfurfurals were very similar to that of 2-furfural itself. All showed a (C=O) stretching frequency at *ca* 1700 cm^{-1} and aldehydic (C-H) stretching frequencies at *ca* 2740 and 2815 cm^{-1}. Absorption bands at 1470 - 1550 cm^{-1} and 1570 - 1600 cm^{-1} were probably attributable to (C=C) stretching of the furan ring. Dimethyl- and trimethylfurfural each showed a weak CH_3 (C-H) stretching band at *ca* 2950 cm^{-1}, while the ethylmethylfurfural also had a band due to CH_2 (C-H) stretching at 2884 cm^{-1}.

An attempt was made to deduce from the infrared data the most likely substitution positions of the dimethyl- and ethylmethylfurfurals (compounds 79, 92 and 103). The IR spectra of authentic samples of 2-furfural and 5-methyl-2-furfural were compared with those of the analogous thiophenes; the overall pattern of absorption bands proved to be very similar. Inspection of the IR spectra for a range of 2-formyl and 2-acetylthiophenes

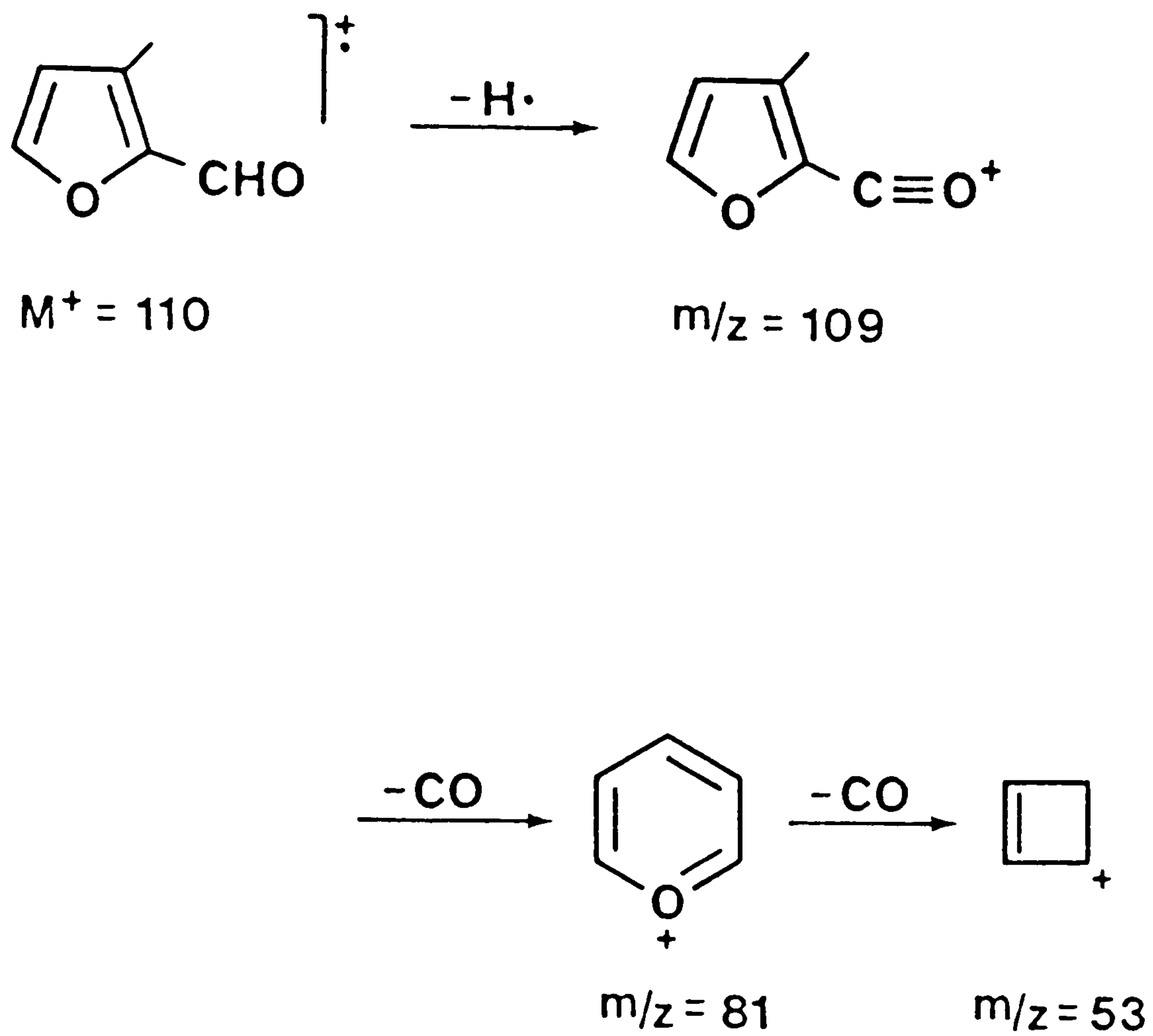


Figure 2.2F: Suggested fragmentation of 3-methyl-2-furfural.

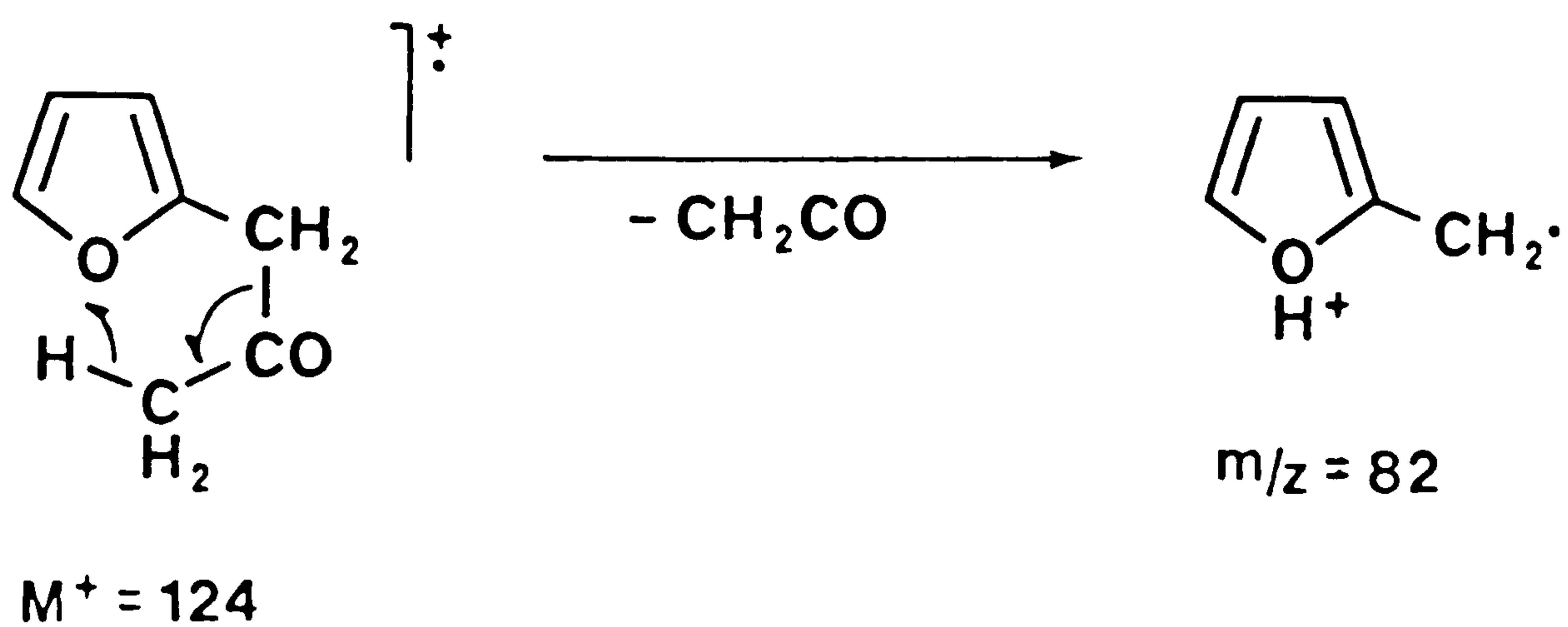


Figure 2.2G: Proposed mechanism for the formation of fragment m/z = 82 from 1-(2-furyl)-2-propanone.

suggested that the wavenumber of the peak at 1400 - 1460 cm^{-1} (corresponding to that at 1470 - 1550 cm^{-1} in furfurals) is raised by *ca* 40 cm^{-1} by a methyl group in the 5 (but not 3 or 4) position. The same effect could be observed on comparing not only 5-methyl-2-furfural with 2-furfural, but also compounds **79**, **92** and **103**. It seems probable, therefore, that at least one of the substituents of these compounds occupies the 5 position. Further study of the acetyl and formylthiophenes indicated that, while the addition of any methyl group causes a drop in the wavenumber of the (C=O) stretching frequency (*ca* 1700 cm^{-1}), the greatest drop was caused by addition in the 3 position; presumably a weak interaction occurs between the methyl group and the carbonyl oxygen. Within the series, 2-furfural, 5-methyl-2-furfural, dimethylfurfural and trimethylfurfural, the addition of the first, second and third methyl groups caused consecutive reductions in the (C=O) stretching frequency of 6, 9, and 3 cm^{-1} respectively. Thus, the most probable substitution pattern for the dimethylfurfural (**79**) is 3,5-dimethyl-2-furfural .

The aldehydic (C-H) and (C=O) frequencies were very similar in the dimethyl and ethylmethylfurfurals, suggesting that they were probably substituted in the same positions. However, an ethyl group in the 3 position would be expected to exert a considerable effect on the frequency of the (C=O) band due to intramolecular H-bonding, so the most likely formula for this compound is 5-ethyl-3-methylfurfural.

The identification of the major headspace constituents, 2-acetylfuran (**60**) and 2-propionylfuran (**72**) was easily achieved by comparison of the mass spectrum and LRI with those of the authentic material. The mass spectrum for compound **78** (LRI = 1601) was analogous to that of 2-acetylfuran and matched well with that of 2-acetyl-5-methylfuran (LRI = 1621); an identity of either 2-acetyl-3 or 4-methylfuran is proposed.

The presence of m/z 43 and the loss of a neutral fragment of the same mass suggest that compound **63** (MW = 124) contains an acetyl group, while m/z 81 and m/z 53 are typical of a furfuryl moiety. Thus, this compound is almost certainly 1-(2-furyl)-2-propanone, an identity corroborated by the mass spectral data cited by Stoll *et al* (1967). The fragment m/z 82 may be due to the loss of ketene by a similar mechanism to that described for 2-pentylfuran (Vernin

and Petitjean 1982) (Figure 2.2G). Compounds 93 (MW = 124)and 94 (MW = 152) probably belong to the same group of furans but have yet to be identified.

Two of the above compounds (2-acetylfuran and 1-(2-furyl)-2-propanone) have been found as products of the reaction between cysteine and ribose (Mulders 1973) and a number have been reported in foods (van Straten and Maarse 1988): furfural, 2-acetylfuran and 2-propionylfuran are constituents of many foods (including heated meats) while 4-methyl-2-furfural, 2-acetyl-4-methylfuran and dimethylfurfural have been found only in rum. 1-(2-Furyl)-propan-2-one has been noted in the volatiles from heated beef and pork as well as some other foods; trimethylfurfural has only been reported in heated beef.

2-Furfural is known to be a breakdown product of pentose sugars, via 1,2-enolization of the Amadori product (Sec. 1.1.1.2; Hodge 1967). However, the formation of the 6,7 and 8 carbon furan derivatives cannot be explained so easily. Possible routes of formation of such sugar-derived compounds are discussed in Section 2.2.2.

Pyrazines

Compound	MW	LRI	No.
Methylpyrazine	94	1271	21
Ethylpyrazine	108	1334	32
2,5-Dimethylpyrazine	108	1328	31
2,6-Dimethylpyrazine	108	1334	33
2,3-Dimethylpyrazine	108	1352	34
2-Ethyl-6-methylpyrazine	122	1386	38
2-Ethyl-5-methylpyrazine	122	1394	39
2-Ethyl-3-methylpyrazine	122	1407	40
Trimethylpyrazine	122	1412	41
2,5-Diethylpyrazine	136	1461	50
2-Ethyl-3,6-dimethylpyrazine	136	1449	46
2-Ethyl-3,5-dimethylpyrazine	136	1466	51
Tetramethylpyrazine	136	1483	54
3,5-Diethyl-2-methylpyrazine	150	1496	59
2-(1-Propenyl)-pyrazine	120	1546	68
Unknown pyrazines (mixture)	150	1517	65

Fourteen pyrazines were clearly identified by comparison with the authentic materials; a further compound was tentatively identified as 3,5-diethyl-2-methylpyrazine while a pair of co-eluting isomeric pyrazines remain unidentified. The formation of these compounds by Strecker degradation is well known and involves the condensation of 1,2-dicarbonyl compounds with amino acids (Dawes and Edwards 1966; Mauron 1981). Many of these pyrazines have been reported previously as products of Maillard systems involving glycine + glucose (Dawes and Edwards 1966), cysteine + ribose (Mulders 1973) or sugars + ammonia (Shibamoto and Bernhard 1977) and they have all been cited as constituents of a number of foodstuffs (van Straten and Maarse 1989).

Comparison of the pyrazines listed above with those obtained from the aqueous reaction of glycine with glucose (Wong and Bernhard 1988) indicates good general agreement; both studies found that 2,5-dimethyl-, 2,6-dimethyl-, trimethyl- and 2-ethyl-3,5-dimethylpyrazines were among the most abundant. These authors rationalise the large quantities of these products in terms of the α-aminocarbonyl intermediates required for their formation. Shibamoto and Bernhard (1977) proposed ten such intermediates and showed how they could be formed from hexose and pentose sugars and ammonia. Of the pyrazines detected from the reaction of glycine with ribose, only 2,5-diethyl-, tetramethyl- and 3,5-diethyl-2-methylpyrazines could not be formed from these fragments. Wong and Bernhard (1988) suggest that tetramethylpyrazine can be formed from the condensation of $HOCH_2CH(NH_2)CHO$ with $CH_3COCH(NH_2)CH_3$, the Amadori product of acetoin, but are unable to suggest plausible mechanisms for the formation of the diethylpyrazines or 2,3-diethyl-5-methylpyrazine. It is possible that the condensation of more complex amino carbonyl compounds, as recently proposed by Yuan *et al* (1989a,b), may offer an explanation for the formation of these pyrazines.

Pyrroles

A mechanism has been proposed (Rizzi 1974) by which 2-acyl-1-methylpyrroles can be formed from amino acids and 2-acylfuran, and it seems likely that 2-formyl-1-methylpyrrole (82) and 2-acetyl-1-

Compound	MW	LRI	No.
2-Formyl-1-methylpyrrole	109	1618	82
2-Acetyl-1-methylpyrrole	123	1654	88
1-(2-Furfuryl)-pyrrole	147	1824	109
Unknown pyrrole	137	1629	84
Unknown pyrrole	151	1665	90
Unknown pyrrole	151	1697	95
Unknown pyrrole	151	1760	102
Unknown pyrrole	165	1720	98

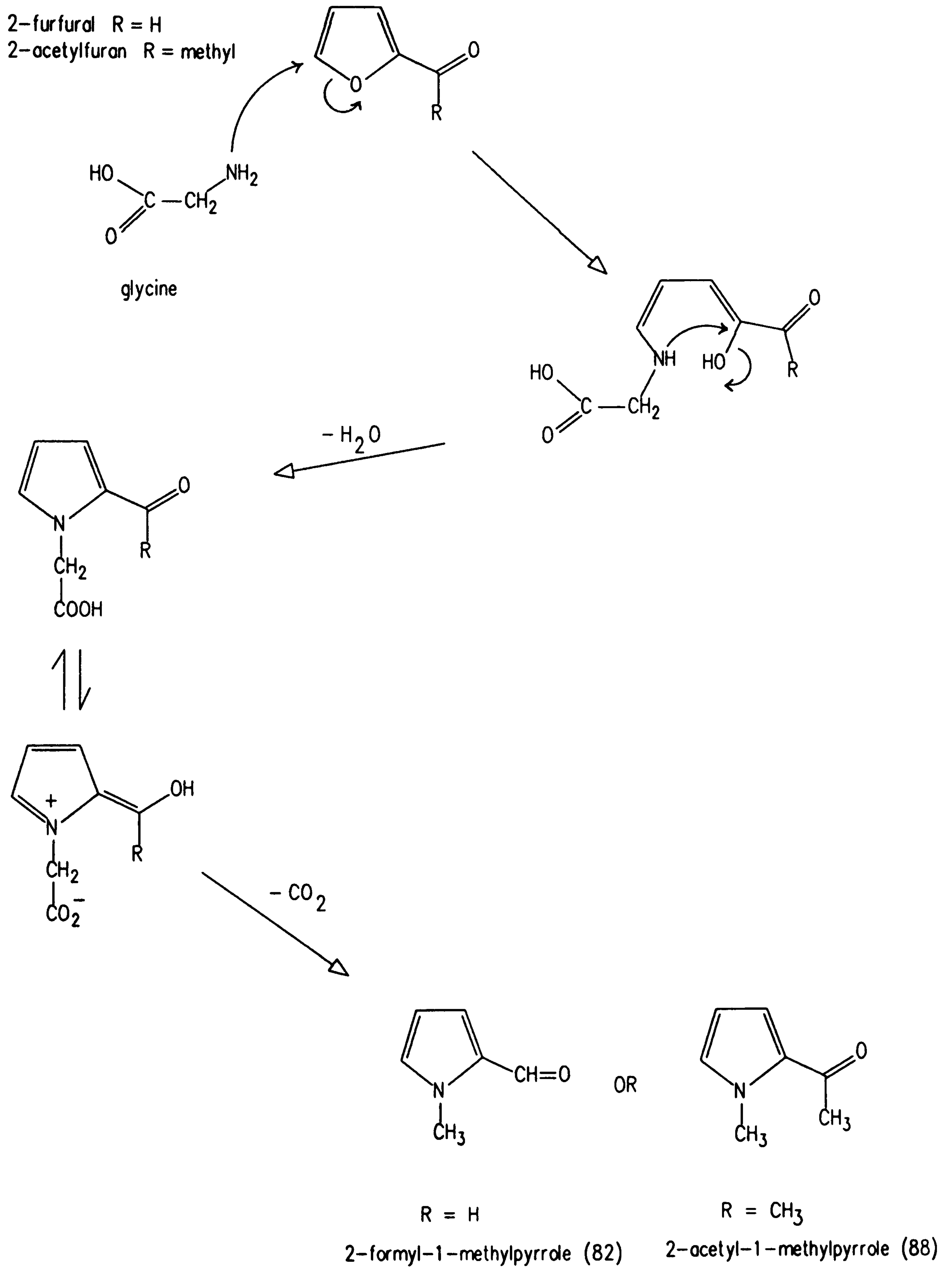
methylpyrrole (**88**) are formed from 2-furfural and 2-acetylfuran by this route, as illustrated in Figure 2.2H. An alternative mechanism of formation from the 1,2-diketo derivative of the Amadori product (Kato and Fujimaki 1968) would involve the reaction of glycine with an earlier stage in the pathway leading to furfural formation, but would yield similar products. It is possible that some of the other pyrroles (**84**, **90**, **95**, **98**, **102**) were also 2-acyl-1-methylpyrroles, formed from the abundant 2-acylfurans described above or their Amadori precursors. The mass spectra of all five of these compounds showed similar fragmentation patterns and were clearly related. Almost identical spectra were obtained for compounds 95 and 102; the fragmentation pattern below ion m/z 109 was very similar to that of 1- or 5-methyl-2-formylpyrrole. It was deduced from the relatively low LRI values that the additional 43 mass units could not contain further oxygen-containing groups, but was more likely to be alkyl in nature, probably shielding the N atom to some extent. Two possible identities emerged as the most probable:

a) 1-butyl-2-formylpyrrole
b) 2-butanoyl-1-methylpyrrole

It is not clear where the butyl substituent of a) could originate from in a lipid-free system. However, while the mass spectrum shows many analogies to that of 2-butanoyl-5-methylfuran the furan does not exhibit the loss of CH_3-CH=CH_2 to give the free formyl group. These compounds warrant further investigation to fully elucidate their structures and aroma properties.

The production of pyrroles from model Maillard systems has been reviewed by Maga (1981) who cited examples of the detection of various 1-alkyl-2-acylpyrroles obtained from amino acid + sugar

Figure 2.2H: Formation of 2-formyl-1-methylpyrrole and 2-acetyl-1-methylpyrrole from 2-furfural and 2-acetyl furan (Rizzi 1974)



systems and from the pyrolysis of Amadori compounds. Four pyrroles, including 2-formyl-1-methylpyrrole and 1-(2-furfuryl)-pyrrole were obtained from a cysteine + ribose system (Mulders 1973) and the former was one of many pyrroles identified from the reaction between rhamnose + ammonia (Shibamoto and Bernhard 1978). Those pyrroles identified in the table are volatile constituents of some foods (van Straten and Maarse 1988) and 2-acetyl-1-methylpyrrole is a component of beef aroma (Watanabe and Sato 1972).

Pyridines

Compound	MW	LRI	No.
Pyridine	79	1209	14
2-Methylpyridine	93	1242	18
4-Methylpyridine	93	1320	30
2-Pentylpyridine	149	1583	75
2-Acetylpyridine	121	1597	77

Of the five pyridines listed, four are products of the Maillard reaction (pyridine, 2- and 4-methylpyridines and 2-acetylpyridine); the first three are present only in extremely small quantities. The mechanism of formation of pyridines from the Maillard reaction is not clearly understood although they are known to be formed by thermal treatment of other heterocyclic compounds (such as N-substituted pyrroles or 2-acetylfurans and ammonia) or by condensation of aldehydes with ammonia (Vernin and Parkanyi 1982). The production of 2-pentylpyridine provides an example of the participation of the phospholipid in the Maillard reaction (Whitfield *et al* 1988) as it is believed to be derived from the reaction of ammonia with the lipid breakdown product, 2,4-decadienal (108). Both butyl- and pentylpyridines have been detected from the heating of glycine with beef fat; their levels were related to the proportion of glycine used in the reaction mixture (Ohnishi and Shibamoto 1984). Whether glycine reacts directly with the lipid degradation products or acts as a source of ammonia is not clear.

2.2.2 RIBOSE DEGRADATION PRODUCTS

Of the compounds listed in Table 2.2a, those in the following Table were also detected among the volatile products from the thermal degradation of ribose alone.

No.	Compound	MW	LRI
3	2,3-Pentanedione	100	1055
4	Hexanal	100	1088
7	2,3-Hexanedione	114	1127
8	3,4-Hexanedione	114	1135
11	Heptanal	114	1182
22	Octanal	128	1280
23	3-Hydroxy-2-butanone	88	1286
47	2-Furfural	96	1455
53	3 or 4-Methylfurfural	110	1480
55	Benzofuran	118	1486
60	2-Acetylfuran	110	1498
63	1-(2-Furyl)-2-propanone	124	1513
69	3 or 4-Methylfurfural	110	1549
72	2-Propionylfuran	124	1571
74	Cyclopent-3-ene-1,2-dione	96	1578
78	2-Acetyl-3 or 4-methylfuran	124	1601
79	A dimethylfurfural	124	1607
85	1-(2-Furyl)-3-butanone	138	1639
91	2-Furanmethanol	98	1665
92	An ethylmethylfurfural	138	1681
103	Trimethylfurfural	138	1766

Many of these compounds, including 2-furfural (47), 2-furanmethanol (91), 2,3-pentanedione (3), 3-hydroxy-2-butanone (23) and cyclopent-3-ene-1,2-dione (74) would be expected from the degradation of a pentose sugar (Heyns *et al* 1966; Hodge 1967). However, the mechanism of formation of the hexanediones (7, 8) and many of the furans requires further elucidation. Heyns *et al* (1966) suggested that degradation products of hexoses with more than 6 carbon atoms may arise from the fragmentation of polymeric material. However, an alternative route of formation is from the aldol condensation of 2 and 3 carbon fragments arising from sugar breakdown. Shibamoto and Bernhard (1977) have suggested routes of formation for a number of fragments from pentose and hexose sugars. Olsson *et al* (1981) listed several condensation products of such compounds and commented that the formation of these reaction products is not dependent on whether the sugar is a pentose or a hexose. Shu *et al* (1985c) suggested that the

formation of alkyl-substituted 3(2H)-furanones with more than 6 carbons, from 2,5-dimethyl-4-hydroxy-3(2H)-furanone, may occur by the condensation of sugar degradation products such as 2,3-pentanedione. Further investigation is required to determine the precise mechanisms of formation of some of these compounds from ribose.

2.2.3 AROMA PROPERTIES

The aroma of the entire glycine + ribose reaction mixture after heating was described as "caramel","sweet","burnt sugar" while the addition of phospholipid added "oily" and "chicken-like" notes. While the caramel-like aroma obtained when glycine is heated with various sugars has been observed previously (Kiely *et al* 1960; El'Ode *et al* 1966; Lane and Nursten 1983), the effect of phospholipid on the aroma of such a system has not been studied, although Ohnishi and Shibamoto (1984) noted that the aroma of glycine heated with beef fat was "unpleasant" rather than "cooked". No reference has been found to the individual aromas which make up the overall odour of glycine-containing Maillard systems.

The gas chromatographic separation of the volatile products of the glycine + ribose system yielded a range of aromas . While the detected aromas cannot be assigned unequivocally to the compounds identified in the chromatogram, in some cases they are consistent with the known odours of the compounds. For example, the "caramel,fudge" aroma at 5.8 min (LRI~1060) is in reasonable agreement with the odour reported (Furia and Bellanca 1975; Fors 1983) for 2,3-pentanedione ("sweet,butter,sickly") and the "biscuit, nutty" aromas at 13.5 to 15.0 min (LRI~1340-1390) accord well with those reported (Fors 1983) for ethyl- and dimethylpyrazines ("nutty, roasted"). The mushroom odour at 12.3 min (LRI~1310) may be attributed to the low concentrations of 1-octen-3-one (Cronin and Ward 1971).

Some of the aromas present in the glycine + ribose system were also detected in the reaction mixture containing phospholipid: eg. the early "caramel" aroma, some pyrazine-type aromas eluting at about 14 min (LRI~1360) and the "burnt, curry" aroma at 22.8 min

(LRI~1630). Although the above-mentioned mushroom odour was not detected, the odour of 1-octen-3-one is known (Cronin and Ward 1971) to become metallic in character at higher concentrations, and the increased quantities of this compound formed in the presence of phospholipid are probably responsible for the metallic odour at 12.5 min (LRI~1310). The addition of phospholipid increases the number of aromas detected; the "caramel", "biscuit", "stale" and "nutty" aromas were dominated by "green", "aldehydic" and sometimes "fatty" aromas. The latter class of odours appears to correspond to hexanal, heptanal, octanal and nonanal while an "unpleasant, floral" aroma was observed in the region of 2-pentylfuran ("pungent, fruity, green") (Furia and Bellanca 1975; Fors 1983). Other odours arising from the addition of phospholipid included several described as "floral", "fruity", "sewage, rotting vegetation", "cakes, bread" and two aromas with chicken-like qualities: "mealy, chicken, roasted" at 10.3 min (LRI~1240) and "fatty, chicken" at 22.1 min (LRI~1610).

In addition to the introduction of many aromas, the presence of phospholipid was responsible for the disappearance of certain odours: the mushroom-like and metallic odours at 16.0 and 16.8 min (LRI~1420,1440) may have been masked by the high levels, and resulting strong odours, of octanal, nonanal and other lipid-derived compounds eluting in these regions. However, the "musty, green" odour observed at 20.8 min (LRI~1570) did not occur in the presence of phospholipid and was not obscured by a strong lipid-derived odour; this aroma may correspond to one of the Maillard products reduced in quantity by phospholipid. This is probably also the explanation for the fewer pyrazine-derived "nutty" aromas noted in the presence of phospholipid.

These sensory assessments illustrate the considerable effect of phospholipid in increasing the range and quantity of aroma compounds generated by the Maillard reaction between glycine and ribose, thus altering the nature and complexity of the final odour.

2.3 VOLATILE COMPOUNDS PRODUCED IN MAILLARD REACTIONS INVOLVING CYSTEINE, RIBOSE AND PHOSPHOLIPID: RESULTS AND DISCUSSION.

The principal volatile components in the headspace of the cysteine + ribose and the cysteine + ribose + phospholipid model reaction systems are listed in order of elution in Table 2.3a. The 156 compounds presented were selected as described for the glycine + ribose reaction mixtures (Sec. 2.2). For each compound the linear retention index (LRI), an indication of its relative abundance and the technique(s) used for identification are listed. Authentic compounds were used for identification wherever possible. Mass spectral data, the accurate mass and the major absorptions in the vapour-phase infrared spectrum are given where appropriate (Sec.2.2).

The aliphatic compounds identified include ketones (10), alcohols (9), hydrocarbons (5), aldehydes (5), mercaptoketones (3) and alkanethiols (2), while the wide variety of heterocyclic products comprises thiophenes (34) and thiophenones (6), furans (18), thiazoles (14), pyrazines (8) and oxazoles (4) as well as saturated rings containing 2 or 3 sulphur atoms (12) and a number of bicyclic compounds (18).

For 82 of the compounds listed in Table 2.3a, the identity was clearly established by comparison of the LRI and mass spectrum with those of the authentic material. Tentative identities are suggested for a further 67 volatile products following interpretation of mass spectral and/or infrared data; nine compounds remain unidentified.

Descriptions of the odours eluting from the GC are shown in Figures 2.3A and B against typical gas chromatograms of the volatile products of each reaction mixture; these odour descriptions represent a collation of the assessors' comments and are included only where there was agreement on the presence of the aroma by two or more individuals. Enlarged versions of Figures 2.3A and B and examples of GC-MS ion chromatograms are given in Appendix II.

Some of the results reported in this Section have been the subject of two publications (Farmer *et al* 1989; Farmer and Mottram 1990b)

Table 2.3a: Volatile components obtained from the reaction between cysteine and ribose in the absence and presence of phospholipid

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
1	decane[c]	+[d]	++	1000	MS + LRI	
2	trans-1,3-nonadiene	-	++	1051	MS	
3	hexanal	-	++	1089	MS + LRI	
4	undecane[c]	+	+	1100	MS + LRI	
5	2-butylfuran	+	+	1120	MS + LRI	ten Noever de Brauw et al. 1980
6	4-methyl-3-penten-2-one	+	++	1136	MS + LRI	
7	4,5-dihydro-2-methylthiophene	+	++	1144	MS	ten Noever de Brauw et al. 1980
8	2,5-dimethylthiophene	+	+	1150	MS + LRI	
9	2-heptanone[c]	+	+++	1183	MS + LRI	
10	heptanal	-	++	1183	MS + LRI	
11	dodecane[c]	+	++	1200	MS + LRI	
12	2,3-dimethylthiophene	+	+	1205	MS + LRI	
13	5-ethyl-4-methyloxazole	+	+	1208	MS	Vitzthum and Werkhoff 1974a
14	trimethyloxazole	+	-	1213	MS + LRI	
15	2-pentylfuran[c]	+	+++	1219	MS + LRI	
16	4-ethyl-5-methyloxazole	++	+	1219	MS	Vitzthum and Werkhoff 1974a
17	2-ethyl-5-methylthiophene	+	+	1226	MS + LRI	ten Noever de Brauw et al. 1980
18	1-heptanethiol	-	++	1231	MS + LRI	
19	2-propylthiophene	+	+	1238	MS + LRI	
20	2-methylthiazole	+	+	1242	MS	ten Noever de Brauw et al. 1980
21	3-octanone	-	++	1249	MS + LRI	
22	thiazole	+	+	1251	MS + LRI	
23	1-pentanol	-	++	1254	MS + LRI	
24	4-ethyl-2,5-dimethyloxazole	+	+	1257	MS + LRI	ten Noever de Brauw et al. 1980
25	dihydro-2-methyl-3(2H)-furanone	++	+	1259	MS + LRI	
26	methylpyrazine	+	+	1269	MS + LRI	
27	2-octanone	-	+++	1277	MS + LRI	
28	a methylpentylfuran	-	+++	1282	ms	95, 152(16), 96(11), 43(10), 41(6), 67(4), 68(3), 53(3), 39(3)
29	2-methyl-3-furanthiol	+++	+++	1290	MS + LRI	Evers et al. 1976
30	tridecane[c]	+	++	1300	MS + LRI	
31	4 or 5-methyl-3-furanthiol	+	+	1304	ms	114, 86(48), 85(44), 71(42), 45(28), 39(26), 67(25), 79(23), 113(22), 81(20); 53(17), 58(9), 99(12)

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
32	2-methyl-5-propylthiophene	-	+	1306	MS	
33	2-hexylfuran	-	+++	1314	MS + LRI	ten Noever de Brauw et al. 1980
34	trans-2-heptenal	-	++	1318	MS + LRI	
35	2,5-dimethylthiazole	++	+	1324	MS + LRI	
36	6-methyl-5-hepten-2-one[c]	+	++	1331	MS + LRI	
37	2,5-dimethylpyrazine	+	+	1331	MS + LRI	
38	ethylpyrazine	++	+	1335	MS + LRI	
39	2,6-dimethylpyrazine	+	+	1335	MS + LRI	
40	1-octanethiol	-	++	1335	MS + LRI	
41	2-butylthiophene	-	++	1338	MS + LRI	
42	an ethyl-3-furanthiol	+	+	1346	ms	113, 128(64), 45(23), 39(19), 65(15), 51(11), 85(10), 69(10), 41(8), 114(8)
43	3-mercapto-2-pentanone	+++	+++	1350	MS, AM	Hartman et al. 1984b AM = 118.0453: $C_5H_{10}OS$
44	1-hexanol	-	+++	1356	MS + LRI	
45	2-methyl-2-cyclopenten-1-one	++	++	1367	MS	
46	4-mercapto-4-methyl-2-pentanone	-	+	1371	MS + LRI	
47	4,5-dimethylthiazole	++	++	1377	MS + LRI	
48	trimethylthiazole	+++	+++	1385	MS + LRI	
49	nonanal	-	+++	1389	MS + LRI	
50	2-ethyl-5-methylpyrazine	++	+	1393	MS + LRI	
51	5-ethyl-2-methylthiazole	+++	++	1403	ms	71, 127(97), 112(83), 85(58), 45(54), 86(49), 59(24), 98(22), 53(20), 58(19)
52	2-ethyl-3-methylpyrazine	++	++	1405	MS + LRI	
53	2-furanmethanethiol	+++	+++	1417	MS + LRI	
54	2-heptylfuran	-	+++	1420	MS + LRI	ten Noever de Brauw et al. 1980
55	a methylvinylthiophene	+	++	1425	ms	124, 123(72), 97(55), 45(23), 39(17), 79(16), 91(14), 125(12), 77(11), 51(7); 109(1)
56	4,5-dimethylisothiazole	+	+	1427	MS	
57	2-pentylthiophene	-	+++	1438	MS + LRI	
58	5-ethyl-4-methylthiazole	+++	++	1440	MS + LRI	
59	1-octen-3-ol	-	+++	1451	MS + LRI	
60	5-ethyl-2,4-dimethylthiazole	+++	++	1451	MS + LRI	

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
61	2-furfural	++	+	1453	MS + LRI	
62	1-heptanol[c]	+	+++	1460	MS + LRI	
63	2,5-diethylpyrazine	+	+	1461	MS + LRI	ten Noever de Brauw *et al*. 1980
64	2-ethyl-3,5-dimethylpyrazine	+	+	1466	MS + LRI	
65	*cis*-dihydro-2,(4 or 5)-dimethyl- 3(2H)-thiophenone	+++	++	1478	ms, AM, ir	60, 130(46), 59(27), 45(18) 61(15), 42(14), 41(14), 39(10), 55(8), 102(6); 74(2), 88(3) AM = 130.0454:$C_6H_{10}OS$ 2977cm^{-1}(m), 2936(w), 1751(s), 1455(w), 1263(w), 1187(w)
66	*trans*-dihydro-2,(4 or 5)-dimethyl-3(2H)-thiophenone	+++	+++	1482	ms, AM, ir	60, 130(41), 59(27), 45(18), 41(16), 42(15), 39(14), 61(14), 55(7), 102(6); 74(2), 88(2) AM = 130.0442:$C_6H_{10}OS$ 2978cm^{-1}(m), 2937(w), 1751(s), 1456(w), 1263(w), 1191(w)
67	an ethylmethylthiazole	+	+	1489	ms	112, 84(88), 54(84), 71(58), 127(50), 45(28), 39(25), 42(22), 41(14), 85(8)
68	2-decanone	-	+++	1490	MS + LRI	
69	2-ethyl-1-hexanol	++	+	1493	MS + LRI	
70	2-acetylfuran	++	+	1496	MS + LRI	
71	1-mercapto-3-pentanone	++	++	1501	ms	57, 61(74), 85(49), 43(31), 118(29), 55(20), 56(19), 47(18), 41(17), 58(13) AM = 118.0449: $C_5H_{10}OS$
72	1-(2-furyl)-2-propanone	+++	+++	1512	MS	Stoll *et al*. 1967
73	2-octylfuran	-	+++	1519	MS + LRI	ten Noever de Brauw *et al*. 1980
74	dihydro-2-methyl-3(2H)-thiophenone	+++	+++	1522	MS + LRI	ten Noever de Brauw *et al*. 1980
75	dihydro-4(5)-methyl-3(2H)-thiophenone	+++	++	1528	ms	46, 116(56), 41(29), 74(24), 42(23), 39(21), 45(19), 47(17), 54(8), 88(7)
76	2-methyl-(4 or 5)-propenyl-thiazole	++	+	1545	ms	97, 139(46), 98(31), 45(18), 71(12), 39(12), 59(11), 57(11), 138(9), 58(7)
77	2-hexylthiophene	-	+++	1547	MS + LRI	

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
78	2,3-dimethyl-2-cyclopenten-1-one	+++	++	1548	MS	ten Noever de Brauw *et al*. 1980
79	dihydro-3(2H)-thiophenone	+++	+++	1556	MS + LRI	
80	2-thiophenethiol	+++	+++	1562	MS + LRI	
81	1-octanol	-	+++	1567	MS + LRI	
82	2-propionylfuran	+++	++	1570	MS + LRI	
83	*cis*-3,5-dimethyl-1,2-dithiolan-4-one	+++	+++	1574	MS	Fohlisch and Gottstein 1979; 60, 148(69), 59(48), 55(29), 92(27), 64(27), 61(16), 45(16), 56(10), 120(8)
84	unknown MW 178	-	+++	1576	AM	Section 2.2 (73)[e]: $C_{12}H_{18}O$
85	2-pentylpyridine	-	++	1580	MS + LRI	
86	2-methyl-3-thiophenethiol	+++	+++	1584	MS, AM	van den Ouweland and Peer 1975; AM = 129.9923: $C_5H_6S_2$
87	*cis*-3,5-dimethyl-1,2,4-trithiolane	++	+	1586	MS + LRI	
88	dihydro-2(5)-ethyl-3(2H)-thiophenone	+++	+++	1598	ms, ir	74, 41(43), 130(33), 45(16), 46(10), 102(10), 73(8), 55(6), 39(6), 101(5) 2974cm^{-1}(m), 2941(m), 2883(w), 1749(s), 1452(w), 1268(w), 1186(w), 1141(w)
89	*trans*-3,5-dimethyl-1,2-dithiolan-4-one	+++	+++	1604	MS, AM	Fohlisch and Gottstein 1979; 60, 148(72), 59(46), 92(28), 55(27), 64(27), 45(17), 61(16), 56(10), 120(8) AM = 148.0005: $C_5H_8OS_2$
90	*trans*-3,5-dimethyl-1,2,4-trithiolane	++	++	1607	MS + LRI	
91	2-acetyl-5-methylfuran	+	+	1615	MS + LRI	ten Noever de Brauw *et al*. 1980
92	*trans*-2-octen-1-ol	-	+++	1617	MS + LRI	
93	*cis*-2-octen-1-ol	-	+++	1619	MS	
94	3,5-dimethyl-2-cyclohexen-1-one	-	+++	1622	MS + LRI	
95	unknown MW 137	+++	+++	1629	AM	Section 2.2 (84)[e]: $C_7H_7O_2N$
96	unknown MW 176	-	+++	1637	AM	Section 2.2 (86)[e]: $C_{12}H_{16}O$

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
97	2-acetylthiazole	+++	+++	1639	MS + LRI	ten Noever de Brauw *et al.* 1980
98	*cis*-3-ethyl-5-methyl-1,2-dithiolan-4-one	++	++	1653	ms	74, 162(74), 41(71), 60(37), 45(32), 69(32), 59(26), 64(26), 55(23), 92(20); 106(10), 134(4)
99	2-heptylthiophene	-	++	1655	MS + LRI	
100	2-pentylthiapyran	-	+++	1661	ms, AM	97, 98(8), 168(6), 53(3), 45(5) AM = 168.0945: $C_{10}H_{16}S$
101	3-methyl-1,2-dithiolan-4-one	+++	++	1664	MS	Fohlisch and Gottstein 1979; Shu *et al.* 1985a
102	1-nonanol[c]	++	+++	1666	MS + LRI	
103	unknown MW 151	++	++	1666	AM	Section 2.2 (90)[e]: $C_8H_9O_2N$
104,105	unknowns MW 128, 138	+++	+++	1671	AM	85, 45(85), 128(74), 138(57), 59(52), 41(43), 74(35), 71(33), 46(31), 73(30) (mixture) AM = 128.0296: C_6H_8OS; 137.9666: $C_3H_6S_3$
106	a 1-(dimethylfuryl)-2-propanone	++	++	1671	ms	109, 43(68), 152(12), 39(12), 41(7), 110(5), 38(4), 53(3), 85(3), 65(3)
107	3-formylthiophene	++	+++	1672	ms	112, 111(98), 39(59), 45(48), 83(30), 43(25), 58(16), 57(14), 38(13), 113(11)
108	*trans*-3-ethyl-5-methyl-1,2-dithiolan-4-one	++	+	1676	ms	74, 162(75), 41(62), 60(33), 69(21), 64(21), 59(19), 55(18), 92(18), 45(13); 106(12), 134(7)
109	2 formylthiophene	+++	+++	1689	MS + LRI	
110	2-(1-hexenyl)thiophene (*cis* or *trans*)	-	+	1695	ms	123, 110(34), 166(33), 45(21), 79(11), 124(11), 111(9), 39(7), 97(7), 77(6)
111	a formylmethylthiophene	+++	++	1708	ms, AM	126, 125(96), 97(53), 53(33), 45(31), 39(16), 127(11), 57(8), 95(8), 69(7) AM = 126.0156: C_6H_6OS
112	unknown MW 151	+++	++	1714		94, 95(71), 123(71), 39(70), 122(52), 151(51), 67(30), 66(26), 136(25), 57(23)

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
113	2-propionylthiazole	+++	+++	1721	ms, ir	57, 112(98), 113(89), 58(33), 85(29), 84(20), 59(14), 86(11), 114(10), 141(9) 3096cm^{-1}(w), 2990(w), 2951(w), 1706(s), 1481(m), 1397(s), 1335(m), 908(s)
114	2-(1-hexenyl)thiophene (trans or cis)	-	++	1724	ms, AM	123, 166(32), 110(32), 45(14), 124(10), 79(9), 108(5), 137(5), 167(4), 125(3) AM = 166.0785: $C_{10}H_{14}S$
115	2,3-dihydro-6-methyl-thieno[2,3c]furan	+++	+++	1728	MS + LRI AM,ir	Buchi et al. 1971 AM = 140.0314: C_7H_8OS 1241cm^{-1}(s), 2862(w), 1579(m), 1482(m), 1444(m), 1262(m), 1107(s), 921(s)
116	a thienylethanal	++	++	1733	ms, AM	126, 97(72), 45(25), 125(20), 53(19), 69(19), 39(11), 51(10), 70(10), 127(8) AM = 126.0147: C_6H_6OS
117	a 3-acetylmethylthiophene	+++	++	1738	ms	125, 140(53), 43(35), 45(29), 97(29), 53(25), 124(16), 126(9), 46(7), 127(7)
118	a trimethyl-2-cyclohexen-1-one	-	+++	1754	MS	Renold et al. 1974; Heller and Milne 1978
119	2-acetyl-3-methylthiophene	+++	++	1761	MS + LRI	ten Noever de Brauw et al. 1980
120	3-acetylthiophene	+++	+++	1771	MS + LRI	
121	2-acetylthiophene	+++	+++	1777	MS + LRI	
122	2-formyl-5-methylthiophene	+++	+++	1781	MS + LRI	ten Noever de Brauw et al. 1980
123	an ethyl-3(2H)-thiophenone	++	++	1790	ms, AM	72, 128(67), 71(36), 45(18), 100(13), 39(12), 73(6), 129(5), 74(4), 55(4) AM = 128.0315: C_6H_8OS
124	1-(3-thienyl)-2-propanone	++	++	1800	ms, AM	97, 43(62), 98(21), 45(19), 140(15), 53(13), 39(4), 99(4), 69(3) AM = 140.0316: C_7H_8OS
125	2-butanoylthiazole	++	++	1804	ms	43, 112(100), 85(63), 41(40), 155(33), 127(29), 58(28), 71(26), 57(25), 99(20); 86(19), 113(19)

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
126	trans, trans-2,4-decadienal	-	++	1814	MS + LRI	
127	2-formyl-3-methylthiophene	+++	+++	1815	MS + LRI	125, 126(93), 45(44), 97(41), 53(39), 39(17), 69(11), 127(9), 51(7), 70(5)
128	1-(2-furfuryl)-pyrrole	++	++	1822	MS + LRI	
129	1-(2-thienyl)-2-propanone	++	++	1826	ms	43, 97(98), 140(39), 98(25), 45(23), 53(11), 39(5), 99(4), 51(3), 141(3)
130	2-(1-heptenyl)thiophene	-	+	1835	ms	123, 110(45), 125(37), 45(28), 180(25), 97(19), 79(16), 72(14), 113(13), 124(12)
131	2-propionylthiophene	+++	+++	1840	MS + LRI	ten Noever de Brauw et al. 1980
132	cis-3,(5 or 6)-dimethyl-1,2-dithian-4-one	+++	++	1847	ms	60, 162(77), 92(70), 55(56) 120(45), 64(36), 42(36), 41(34), 59(32), 56(25); 74(5), 103(11)
133	3-methyl-1,2,4-trithiane	+++	+++	1857	MS + LRI	
134	thieno[2,3b]thiophene	+++	++	1865	MS + LRI	ten Noever de Brauw et al. 1980
135	3-ethyl-2-formylthiophene	+++	+++	1871	ms, AM, ir	140, 139(41), 111(40), 45(31), 97(29), 125(21), 53(18). 39(17), 123(15), 77(14); 67(8), 69(7), 85(7) AM = 140.0299: C_7H_8OS 2979cm^{-1}(m), 2828(w) 2731(w), 1693(s), 1528(w), 1422(m), 1179(w)
136	thieno[3,2b]thiophene	+++	+++	1874	MS + LRI	
137	trans-3,(5 or 6)-dimethyl-1,2-dithian-4-one	++	++	1876	ms	60, 92(60), 162(59), 55(52), 120(39), 59(37), 41(36), 42(35), 64(31, 56(22); 74(6), 103(9)
138	unknown thiophene MW 152	++	++	1888	ms, AM	124, 123(74), 97(35), 152(28), 125(26), 45(23), 39(19), 91(12), 79(10), 65(9); 109(4); AM = 152.0305: C_8H_8OS
139	3-methyl-1,2-dithian-4-one	+++	+++	1894	MS, AM	ref. 14 AM = 147.9991; $C_5H_8OS_2$
140	a dihydrothienothiophene	+++	++	1910	ms, AM	142, 141(59), 97(58), 96(33), 45(30), 127(22), 69(21), 53(18), 39(16), 143(13); 67(13), 71(11) AM = 141.9890: $C_6H_6S_2$

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
141	a methylthienothiophene	++	++	1918	ms, AM	153, 154(84), 155(17), 69(12), 39(9), 156(9), 77(8), 109(7), 45(5), 121(5) AM = 153.9892: $C_7H_6S_2$
142	a dimethylformylthiophene	+++	++	1924	ms, AM, ir	139, 140(79), 67(16), 111(15), 45(13), 39(11), 77(10), 141(10), 59(8), 71(7) AM = 140.0288: C_7H_8OS 2936cm^{-1}(w), 2806(w), 2728(w), 1691(s), 1448(m), 1383(w), 1196(w)
143	1,2-dithian-4-one	+++	+++	1930	ms, AM, ir	134, 46(65), 55(44), 78(41), 45(38), 60(35), 42(29), 64(27), 106(18), 88(13) AM = 133.9859: $C_4H_6OS_2$ 2924cm^{-1}(w), 1730(s), 1420(vw), 1520(vw)
144	a methylthienothiophene	+++	+++	1940	ms, AM	153, 154(75), 155(16), 69(16), 77(12), 45(12), 39(7), 84(7), 156(7), 109(7); 121(5) AM = 153.9859: $C_7H_6S_2$
145	unknown thiophene MW 166	+++	++	1953	AM	138, 123(54), 137(53), 166(39), 59(31), 39(18), 111(17), 77(17), 45(14), 139(10); 97(8) AM = 166.0453: $C_9H_{10}OS$
146	a dihydrothienothiophene	+++	++	1962	ms, AM, ir	142, 97(74), 141(70), 45(16), 143(14), 144(9), 69(8), 51(8), 58(7), 39(7), 2952cm^{-1}(s), 2859(w), 1728(vw), 1543(w), 1443(m), 1358(m), 1000(m), 832(s), 770(s)
147	thieno[3,4b]thiophene	+++	+++	1976	MS	
148	a methyldihydrothieno-thiophene	+++	+++	1999	ms, AM	156, 155(67), 45(19), 123(18), 59(17), 121(15), 157(14), 111(13), 141(12), 69(9); 77(8), 97(9) AM = 156.0065: $C_7H_8S_2$
149	a dihydrothienothiophene	+++	+++	2022	ms, AM, ir	142, 141(77), 97(55), 45(25), 69(14), 143(14), 144(9), 39(8), 71(7), 140(6); 109(3), 127(3) AM = 141.9958: $C_6H_6S_2$ 2952cm^{-1}(m), 2859(w), 1544(w), 1443(w), 1358(m), 1263(w), 1000(w), 832(m), 789(s)

	Compound	Phospholipid Absent	Phospholipid Present	LRI	Method of identification[a]	MS/IR data[b]
150	a methyldihydrothieno-thiophene	+++	+++	2038	ms, ir	156, 155(59), 111(38), 141(28), 59(24), 97(15), 157(14), 45(14), 39(12), 122(11); 77(10), 121(10) 3072cm^{-1}(w), 2936(s), 2869(m), 1227(w), 1115(w), 814(m)
151	a dimethyldihydrothieno-thiophene	+++	++	2077	ms, ir	170, 169(65), 59(27), 137(17), 155(17), 171(14), 135(14), 172(9), 153(9), 91(9); 77(6), 97(7), 111(8) 2932cm^{-1}(s), 2872(m), 1592(w), 1443(w), 1194(w), 1147(w), 778(w)
152	a methyldihydrothieno-thiophene	+++	+++	2103	ms, ir	156, 155(39), 141(26), 45(16), 59(16), 121(15), 111(14), 123(14), 97(13), 157(12); 77(6) 2943cm^{-1}(s), 2870(w), 1577(m), 1407(m), 1346(m), 1190(m), 776(m)
153	bis(2-methyl-3-furyl) disulphide	+++	++	2152	MS + LRI	Buttery et al. 1984
154	2-methyl-3-furyl 2-methyl-3-tetrahydrofuryl disulphide	+	+	2238	ms	43, 113(53), 230(25), 114(23), 45(22), 73(15), 81(15), 145(15), 41(13), 51(10); 187(6)
155	2-methyl-3-furyl 2 or 3-tetrahydrofuryl sulphide	-	++	2240[f]	ms	113, 85(23), 45(9), 114(7), 41(6), 57(5), 43(5), 115(5), 184(5), 39(4)
156	2-methyl-3-furyl 4 or 5-methyl-3-tetrahydrofuryl disulphide	+	+	2250[f]	ms	57, 113(76), 114(35), 43(28), 230(26), 59(26), 51(12), 45(12), 85(11), 81(10), 145(6), 173(10)

a. Methods of identification

MS + LRI	Mass spectrum and LRI agree with those of authentic compound.
MS	Mass spectrum agrees with literature spectrum (reference given under MS/IR data).
ms	Interpretation of mass spectrum and comparison with those of related compounds.
ir	Interpretation of infrared spectrum and comparison with those of related compounds.
AM	Accurate mass data obtained by high resolution MS (details given under MS data).

b. Mass spectra are given where not previously reported in the literature. The ten most abundant ions are cited in order of decreasing relative intensity (excluding ions <2% base peak) followed by any other ions aiding interpretation. The molecular ion is underlined. For the other mass spectra the literature reference is Heller and Milne (1978) unless otherwise stated.

c. The presence of trace amounts of these nine compounds in the reaction systems containing only cysteine and ribose may be due to cross-contamination from lipid-containing samples, although great care was taken in the cleaning and drying (150°C) of glassware and conditioning of traps (at least 1 hour at 250°C). The amounts found were extremely small compared with the quantities in the lipid-containing systems.

d. Relative size of peaks in GC-MS chromatogram: +++ large; ++ medium; + small; - not detected.

e. Mass spectral data for these compounds have been listed in Section 2.2 (the compound number is given in parentheses).

f. Estimated LRI values.

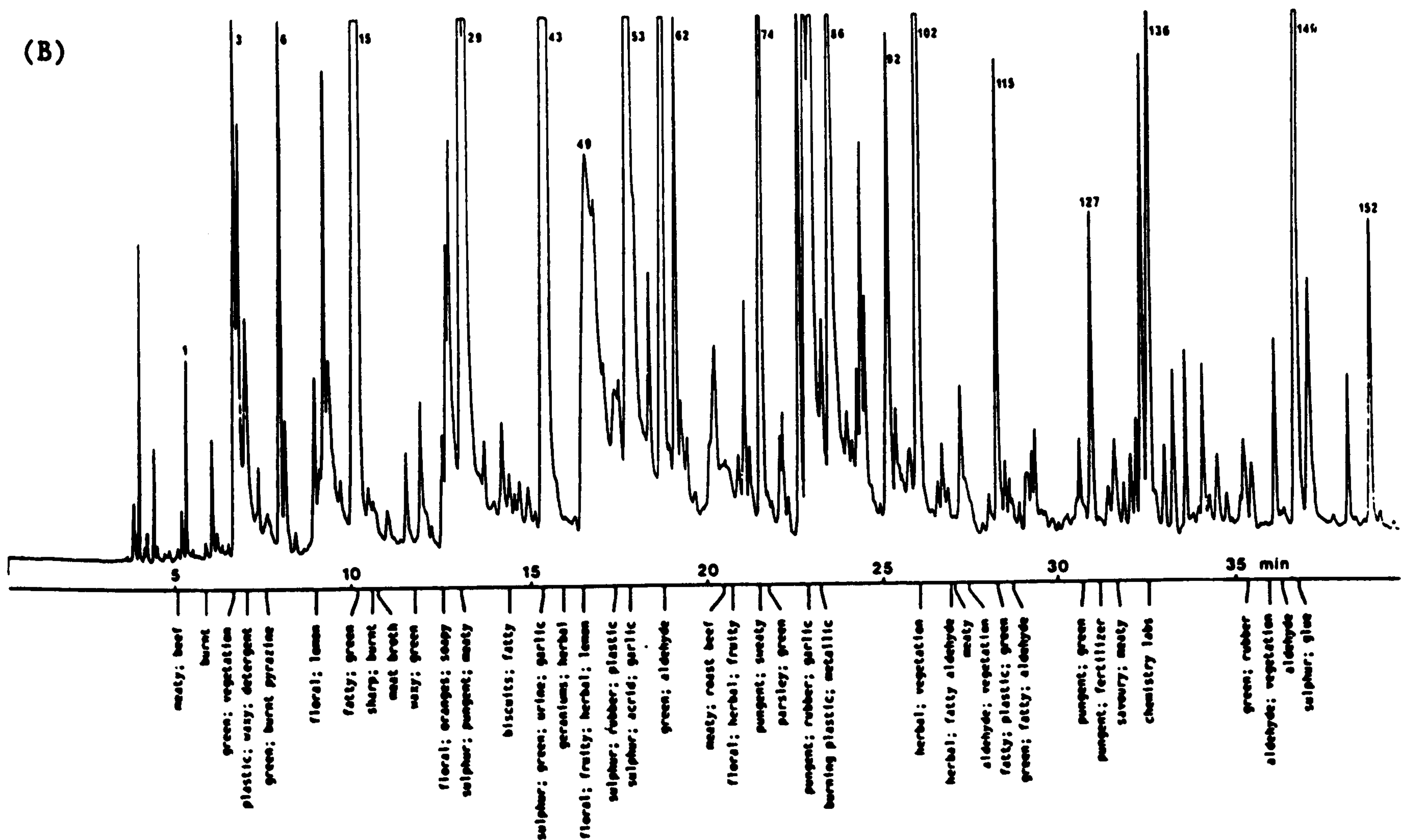


Figure 2.3A and B: Typical gas chromatograms of the volatile products of (A) the reaction between cysteine and ribose and (B) the reaction between cysteine, ribose and phospholipid, showing the positions of selected compounds (numbers refer to Table 2.3a) and a summary of the aromas detected in the column effluent. FID sensitivity: (A) 16 pA full scale (B) 32 pA full scale.

2.3.1 Identification of volatile compounds

The volatile compounds formed in the reaction between cysteine and ribose were dominated by sulphur-containing heterocyclic compounds, particularly certain thiols and thiophenes. Although many of the Maillard reaction products detected in the reaction mixture containing glycine and ribose also occurred in the cysteine-containing system, their quantities were reduced and they comprised a relatively small proportion of the headspace volatiles. The formation of furans, pyrazines and other non-sulphur Maillard products has already been discussed in Section 2.2 and will not be examined further here.

As expected, the presence of phospholipid in the cysteine + ribose Maillard model system added many lipid-derived compounds to the headspace volatiles. These included hydrocarbons, saturated and unsaturated alcohols, aldehydes and ketones formed by the thermal oxidation of the lipid (Grosch 1982; Forss 1982). Of particular interest, however, were those products formed specifically by the interaction of the lipid in the Maillard reaction. Such compounds included various alkyl and alkenylthiophenes, alkanethiols and 2-pentylpyridine. This Section will concentrate on the identification of volatile compounds specific to the reaction of cysteine + ribose and those derived from the interaction of phospholipid, followed by a discussion of the resultant odours and their relevance to the aroma of meat.

Furanthiols, thiophenethiols and their disulphides

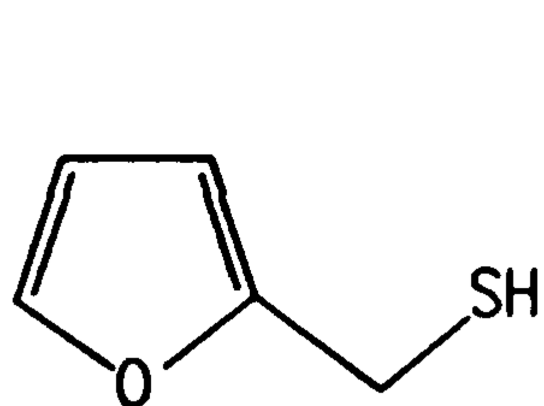
Among the dominant peaks in the early part of the chromatogram of volatiles from cysteine + ribose alone (Figure 2.3A) were 2-furanmethanethiol (53) and 2-methyl-3-furanthiol (29). 2-Furanmethanethiol was probably formed by the reaction of H_2S with the sugar breakdown product, 2-furfural (Shibamoto 1977) and is known to be a component of the aroma volatiles from beef and coffee.

Compound	MW	LRI	No.
2-Furanmethanethiol	114	1417	53
2-Methyl-3-furanthiol	114	1290	29
4 or 5-Methyl-3-furanthiol	114	1304	31
An ethyl-3-furanthiol	128	1346	42
2-Thiophenethiol	116	1562	80
2-Methyl-3-thiophenethiol	130	1584	86
2-Methyl-3-furyl tetrahydrofuryl sulphide	184	2194	155
Bis(2-methyl-3-furyl) disulphide	226	2152	153
2-Methyl-3-furyl 2-methyl-3-tetrahydrofuryl disulphide	230	2238	154
2-Methyl-3-furyl 4 or 5-methyl-3-tetrahydrofuryl disulphide	230	2250e	156

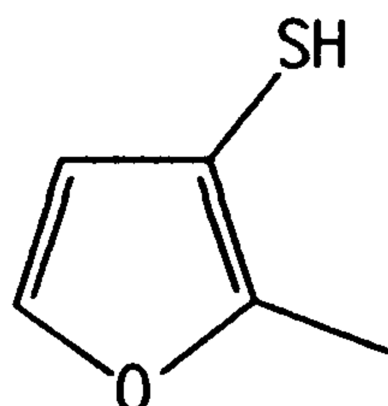
e = estimated

2-Methyl-3-furanthiol is believed to play an important role in the flavour of meat (Gasser and Grosch 1988; Sec. 1.1.3.4), so the occurrence of this compound and a number of related compounds was of particular interest; probable structures of these compounds are shown in Figure 2.3C. Compounds 31 and 42 were tentatively identified as 4 or 5-methyl-3-furanthiol and an ethyl-3-furanthiol. The mass spectrum of compound 31 was almost identical to that of 2-methyl-3-furanthiol, and although the major loss from the ethyl-3-furanthiol (42) was of a methyl radical to give a base peak at m/z 113, below this ion the mass spectrum contained many of the same ions as the methylfuranthiols. 2-Thiophenethiol (80) and a compound identified as 2-methyl-3-thiophenethiol (86) were also among the major volatiles. The mass spectrum of this latter compound compared well with that cited by van den Ouweland and Peer (1975) and the accurate mass data corroborated this identification. The mass spectral fragmentation of this compound differed from that of 2-methyl-3-furanthiol in that the major loss from the thiophene was of an SH radical, whereas that from the furan was of a CO molecule. The most likely route of formation of both the furanthiols and the thiophenethiols is by the reaction of H_2S with sugar-derived furanones (see Fig. 1.1L; van den Ouweland and Peer 1975; Vernin and Parkanyi 1982).

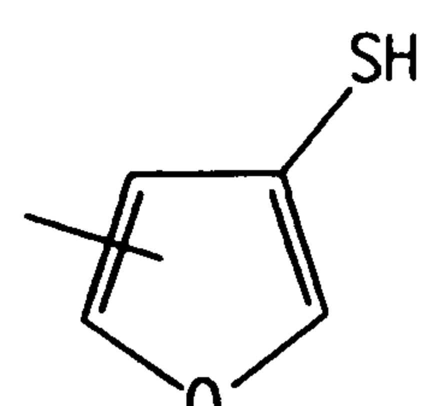
Figure 2.3C: Probable structures of furanthiols, thiophene-thiols, a sulphide and disulphides from the reaction between cysteine, ribose and phospholipid



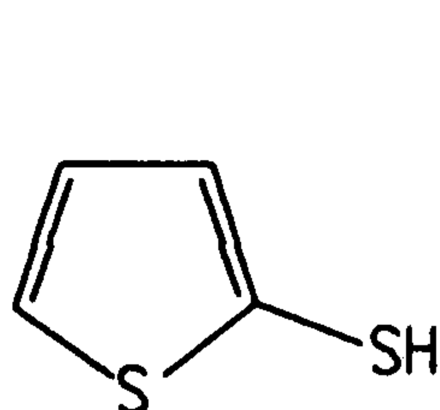
2-furanmethanethiol (53)



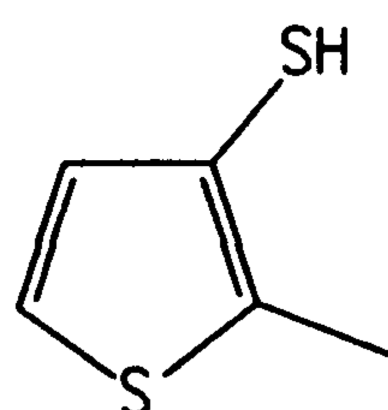
2-methyl-3-furanthiol (29)



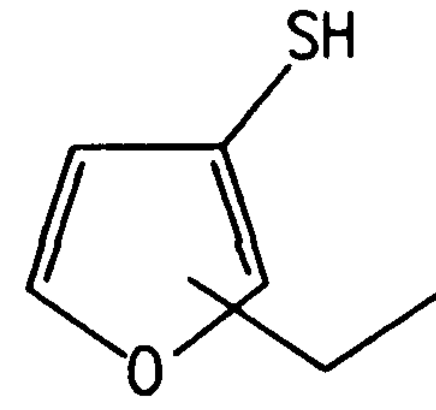
4 or 5-methyl-3-furanthiol (31)



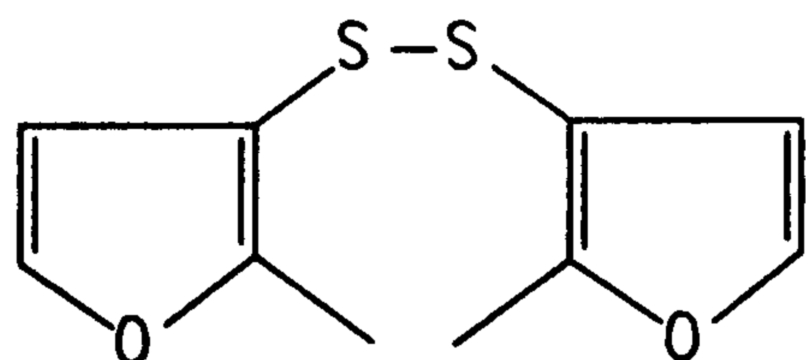
2-thiophenthiol (80)



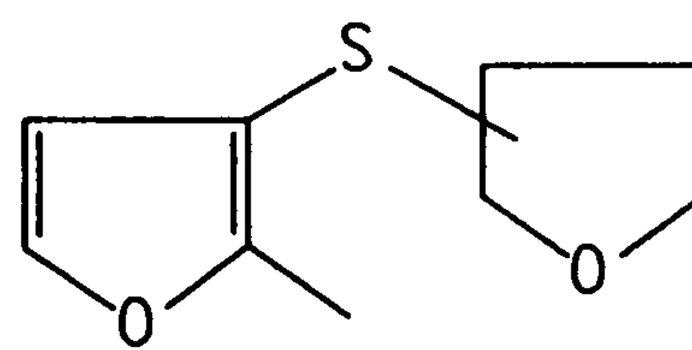
2-methyl-3-thiophenethiol (86)



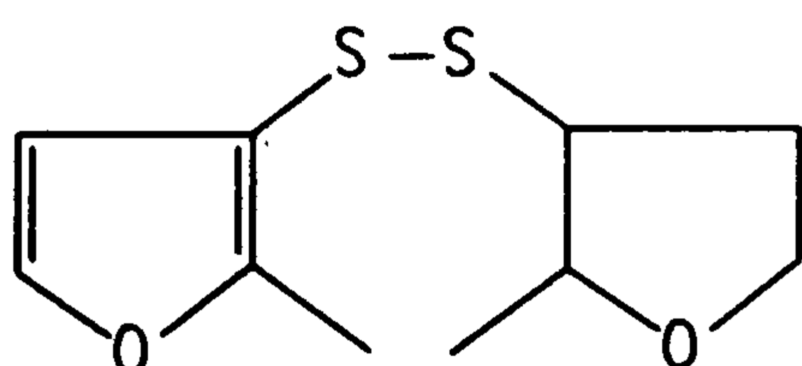
an ethyl-3-furanthiol (42)



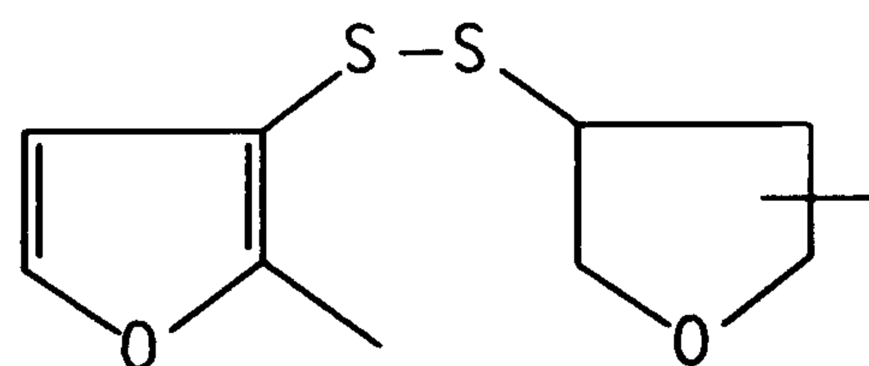
bis(2-methyl-3-furyl) disulphide (153)



2-methyl-3-furyl tetrahydrofuryl sulphide (155)



2-methyl-3-furyl 2-methyl-3-tetrahydrofuryl disulphide (154)



2-methyl-3-furyl 4 or 5-methyl-3-tetrahydrofuryl disulphide (156)

Of special interest was the tentative identification of a number of compounds (**153** - **156**) in which furans were joined by a sulphide or disulphide bridge (Fig. 2.3C). The mass spectra of these substances were dominated by the fragments arising from fission at the (di)sulphide bridge; the predominant ion was that at m/z 113 (2-methyl-3-furylthio ion). Subsequent fragmentation followed closely that of the component thiols.

Gasser and Grosch (1988) have demonstrated that a particularly important contribution to beef odour is made by 2-methyl-3-furanthiol and bis(2-methyl-3-furyl) disulphide; these were among the key odour impact compounds of beef. Both compounds have extremely low odour threshold values, with that for bis(2-methyl-3-furyl) disulphide reported to be 2 parts in 10^{14} of water (Buttery 1984), one of the lowest odour thresholds yet reported. 2-Methyl-3-thiophenethiol has also been reported to possess a "roast meat" aroma (van den Ouweland and Peer 1975) and it seems possible that the sulphides and disulphides of this compound and 2-methyl-3-furanthiol may contribute to meat flavour. Recent investigations have reported the presence of such compounds in the volatiles from cooked beef (Farmer and Patterson 1990; Werkhoff *et al* 1990).

Of particular interest was the observation that compound **155** occurred only in the presence of lipid. Many compounds containing the 2-methyl-3-furylthio- moiety have been shown to have intense and distinctive aromas (van den Ouweland and Peer 1975; Werkhoff *et al* 1990); the occurrence of lipid-dependent members of this compound class may contribute to the increased meaty character of the phospholipid-containing Maillard reaction.

Acylthiophenes

Many of the large group of acylthiophenes were directly analogous to the acylfurans generated by the glycine + ribose reaction (Section 2.2). Several 2-acylthiophenes (compounds **109**, **119**, **121**, **122**, **127**, **131**) were readily identified by comparison with authentic samples; they have also been reported in the volatiles

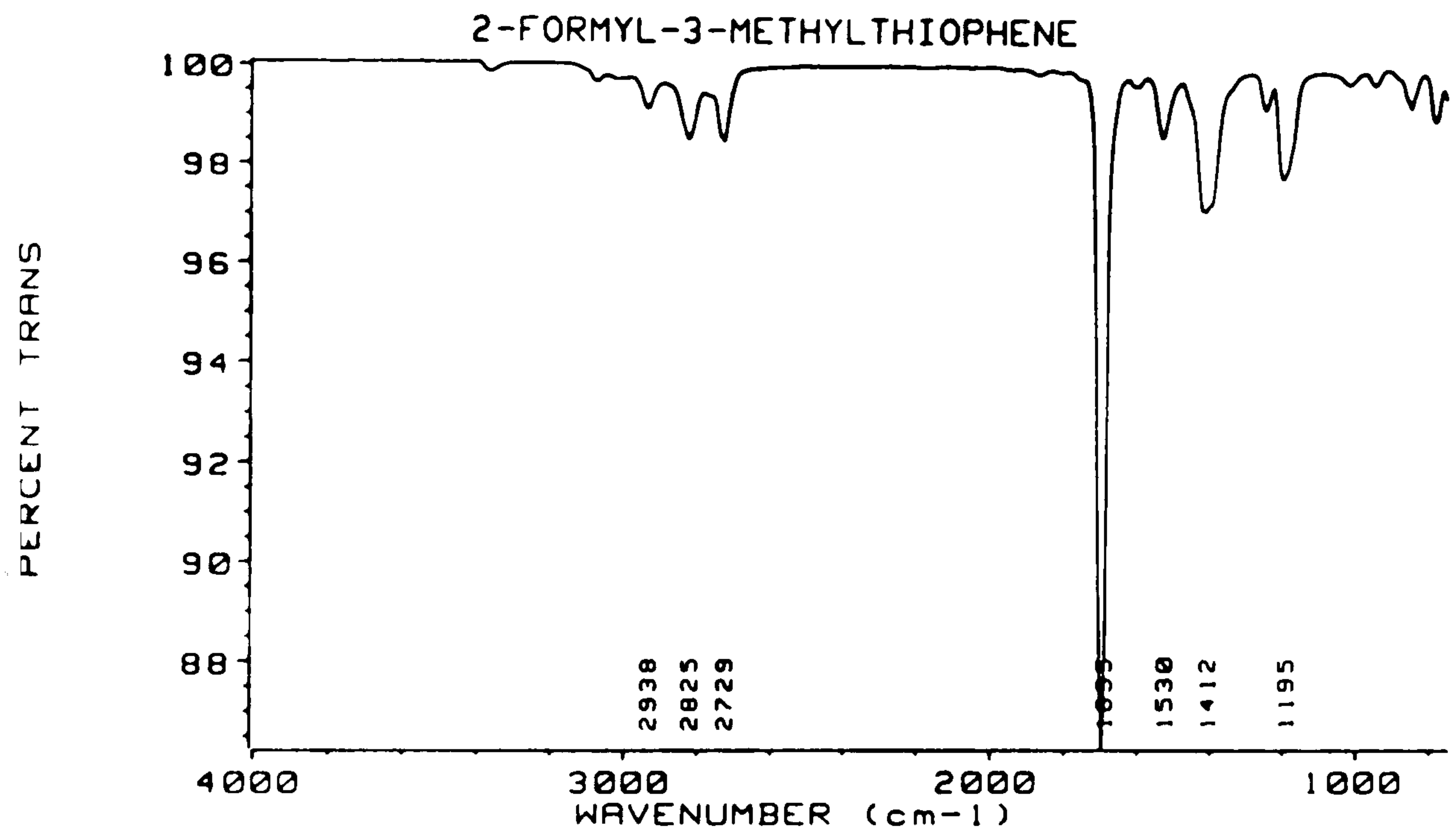
Compound	MW	LRI	No.
3-Formylthiophene	112	1672	107
2-Formylthiophene	112	1689	109
2-Formyl-5-methylthiophene	126	1781	122
2-Formyl-3-methylthiophene	126	1815	127
3-Ethyl-2-formylthiophene	140	1871	135
A formylmethylthiophene	126	1708	111
A dimethylformylthiophene	140	1924	142
3-Acetylthiophene	126	1771	120
2-Acetylthiophene	126	1777	121
2-Acetyl-3-methylthiophene	140	1761	119
A 3-acetylmethylthiophene	140	1738	117
2-Propionylthiophene	140	1840	131
1-(3-Thienyl)-2-propanone	140	1800	124
1-(2-Thienyl)-2-propanone	140	1826	129
A thienylethanal	126	1733	116

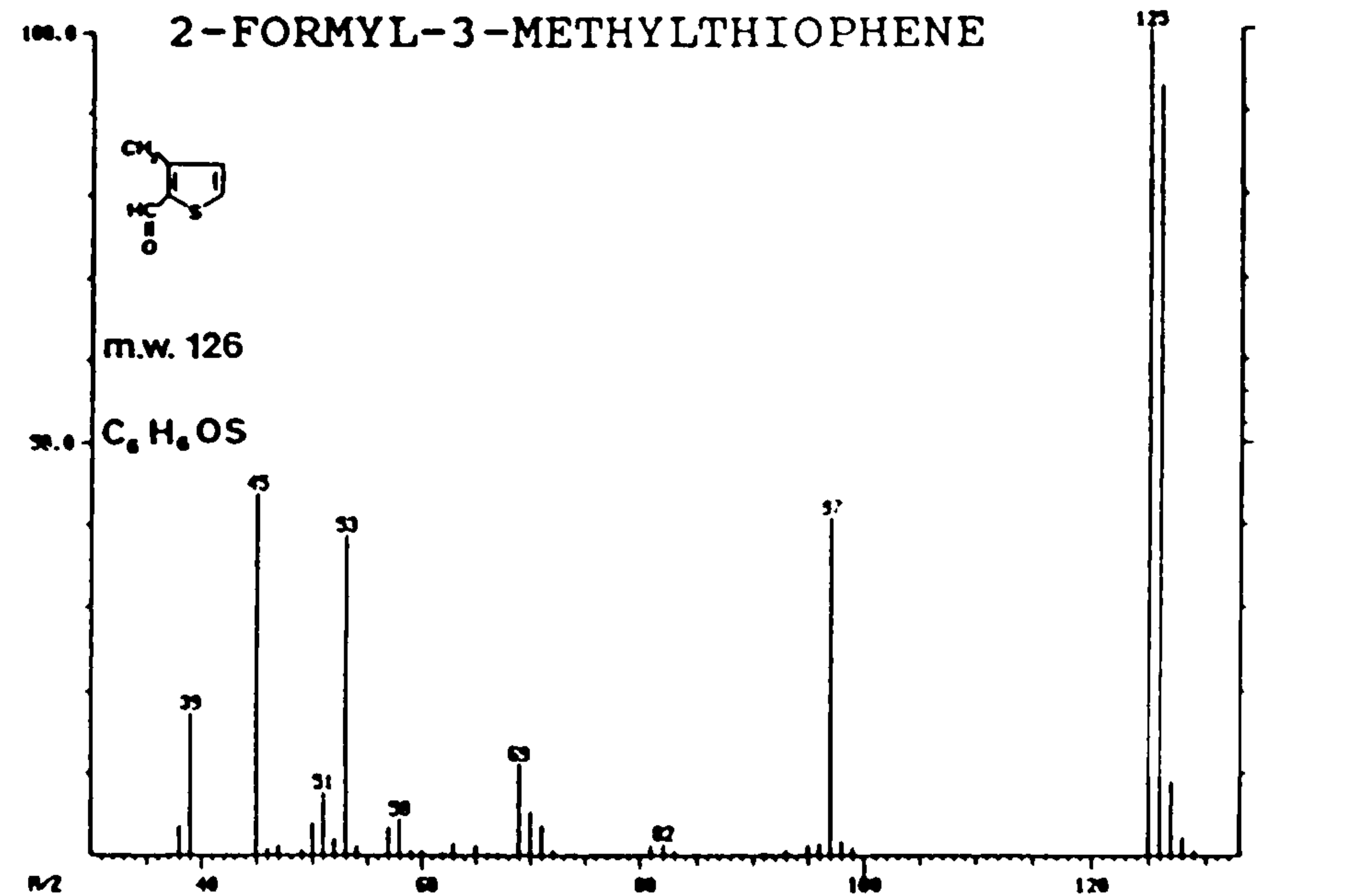
from a number of foods, especially coffee and cooked meats (van Straten and Maarse 1988).

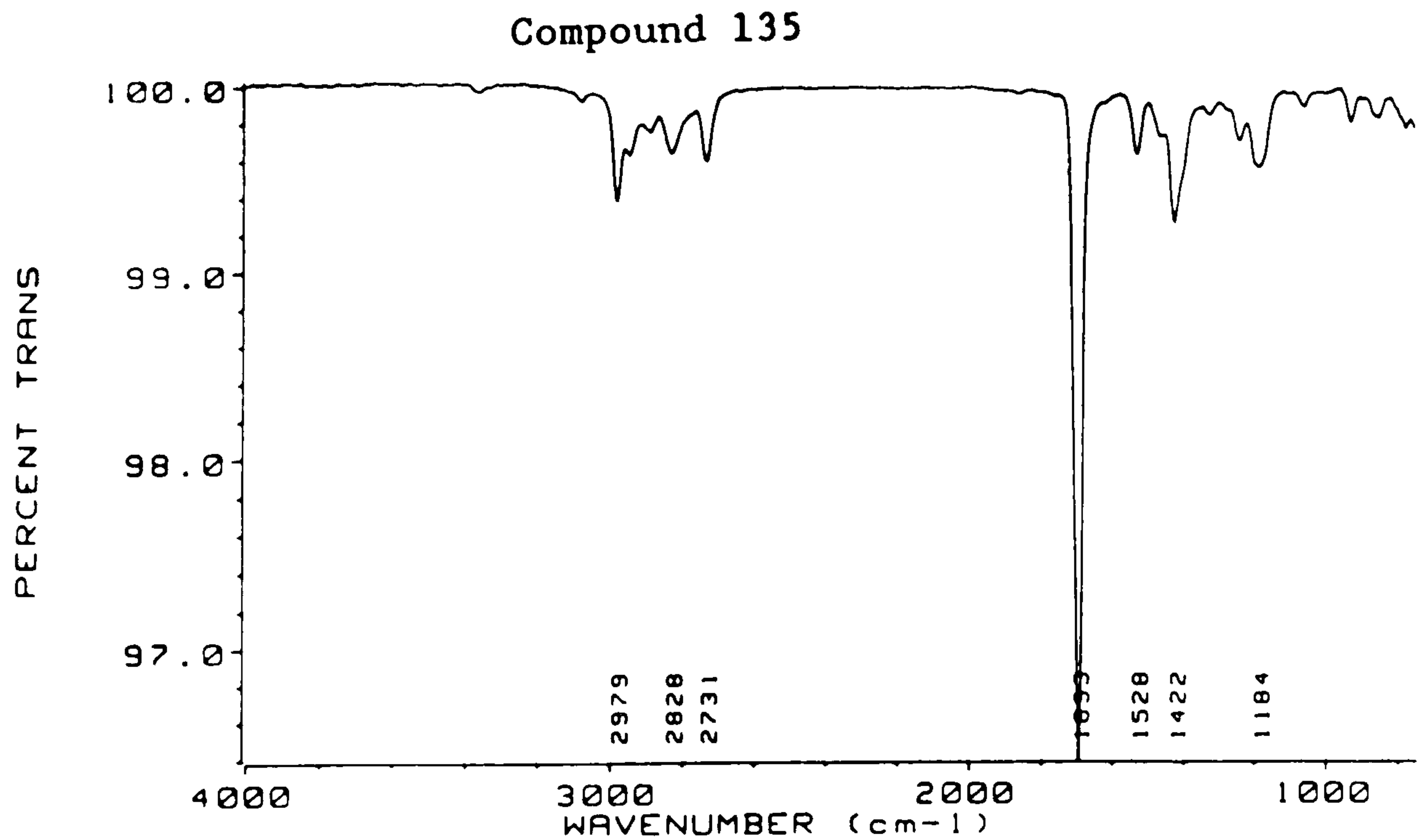
An identity of 3-ethyl-2-formylthiophene is suggested for compound **135**; the peaks of the infrared spectrum matched well with those of 2-formyl-3-methylthiophene, except for the somewhat larger saturated C-H absorption at 2979cm^{-1} (see Fig. 2.3D). The molecular formula and mass spectrum are consistent with this identity; abundant ions resulted from the loss of hydrogen (m/z 139) or methyl (m/z 125) radicals, followed by the loss of CO (m/z 111, 97), then CS (m/z 67, 53).

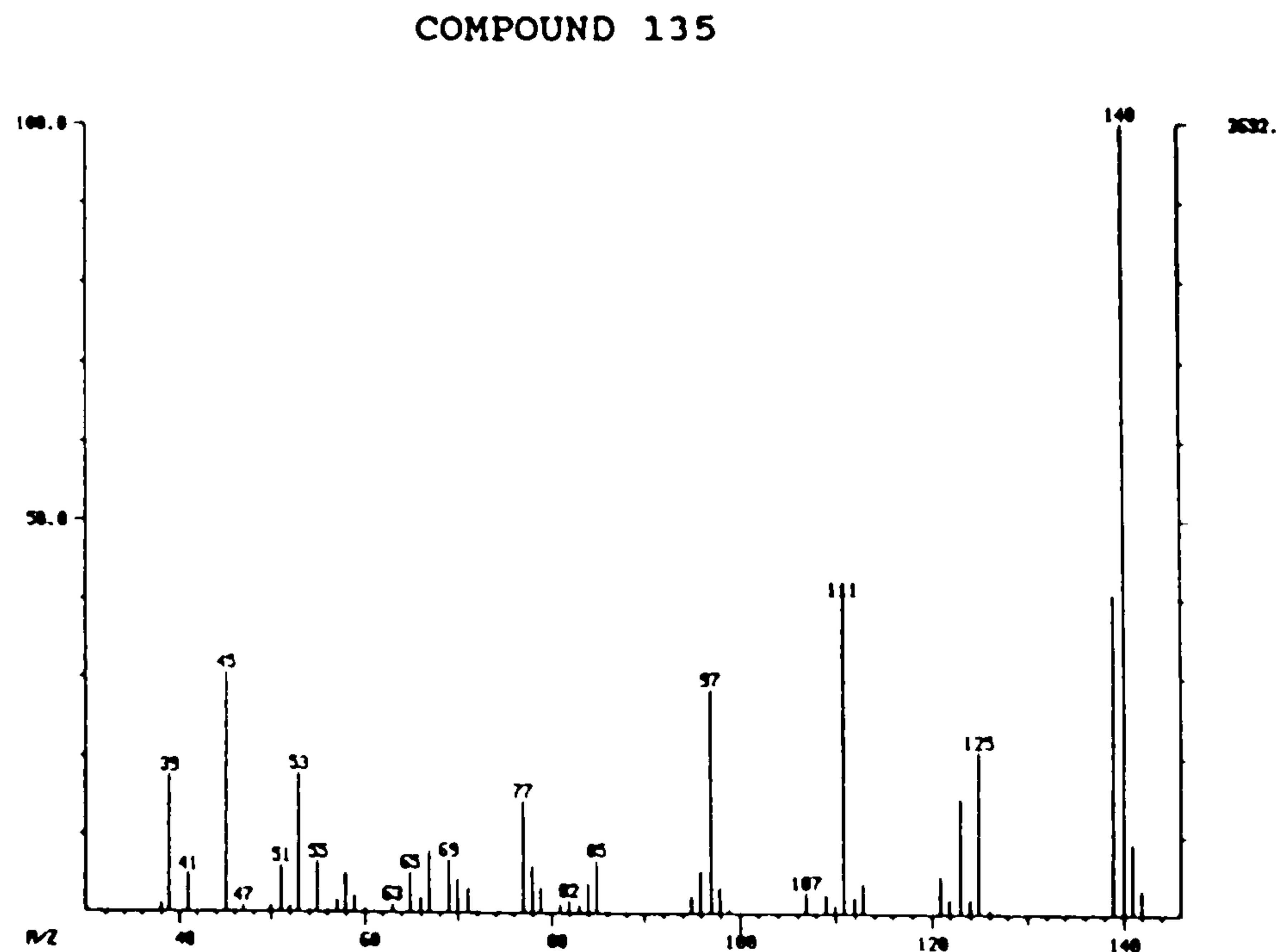
3-Acylthiophenes were also identified (**107**, **117**, **120**) although an authentic reference compound was only available for 3-acetylthiophene. These compounds eluted just before the corresponding 2-acyl isomer and in each case the mass spectrum showed a slightly larger ion resulting from the loss of the formyl or acetyl group. The mass spectra of equivalent thiophenes and furans often show considerable similarities, which aided the identification of a number of compounds. In particular, compounds **124** and **129** both showed mass spectra analogous to that of 1-(2-furyl)-2-propanone; it seems probable that the earlier isomer is the 1-(3-thienyl)-2-propanone and the later one, 1-(2-thienyl)-2-

Figure 2.3D: Infrared spectra of authentic 2-formyl-3-methylthiophene and compound 135 (suggested identity : 3-ethyl-2-formylthiophene).









propanone. Again, the ion resulting from the loss of an acetyl radical was more dominant in the earlier spectrum.

The low $M-1^+$ ion in the mass spectrum of compound 116 suggested that, despite other similarities to the spectra of the formylmethylthiophenes, the formyl group was not attached to the ring in this case; inspection of mass spectral data for furans with an aldehyde group alpha to the ring (Heller and Milne 1978) indicated that they do not show either the large $M-1^+$ ion characteristic of a ring formyl group or the loss of water, but readily lose the entire CHO group. The mass spectrum of compound 116 showed such a loss to give an abundant ion at m/z 97. Thus, a thienylethanal is proposed.

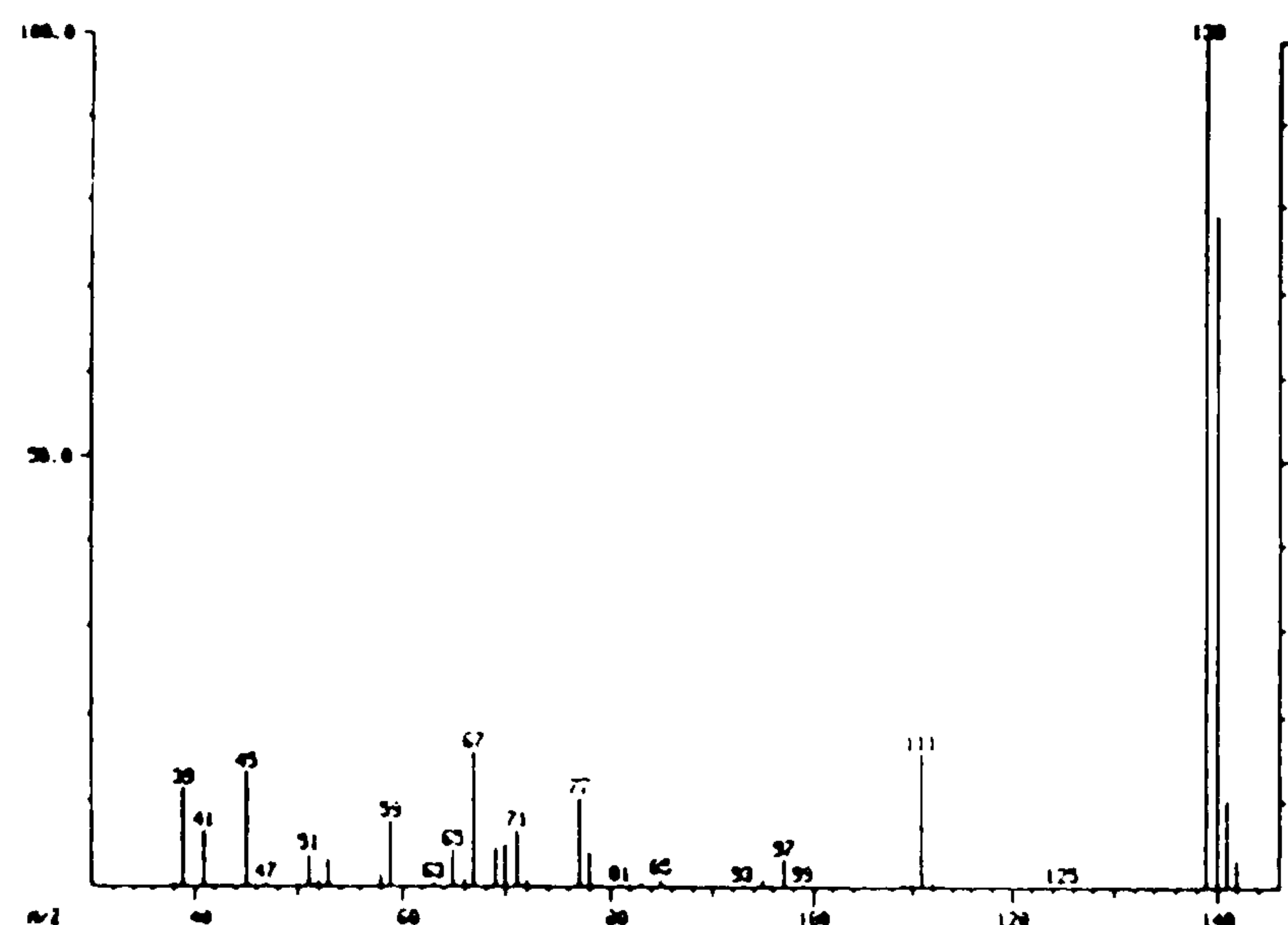
Compound 111 was clearly identified as a formylmethylthiophene by its mass spectrum; however, as the mass spectra of isomers of this compound are so similar, it is not possible to deduce the exact substitution pattern. The fact that this compound elutes considerably earlier than the 2-formyl-5-methyl- and 2-formyl-3-methylthiophenes may suggest that it is a 3-formylmethylthiophene rather than the 2-formyl-4-methylthiophene.

A dimethylformylthiophene (compound 142) was identified from its accurate mass data and by comparison of its mass spectrum with that of the analogous dimethylfurfural identified in the volatile products of the Maillard reaction between glycine and ribose (compound 79 in Sec. 2.2). Like the dimethylfurfural, the major fragments were caused by the consecutive loss of the aldehydic H radical to give m/z 139, CO (m/z 111) and CS (m/z 67). The loss of H_2S from m/z 111 gave an ion at m/z 77, in contrast to the dimethylfurfural for which the analogous loss of H_2O gave only a minor fragment. The infrared spectra of these two compounds also showed clear similarities (see Fig. 2.3E). Compound 142 gave the following absorption bands (corresponding bands for dimethylfurfural are given in brackets): 2936 cm^{-1} (2940) due to methyl (C-H) stretching; 2728, 2806 cm^{-1} (2737, 2819) due to aldehydic (C-H) stretching frequencies; 1691 cm^{-1} (1699) from (C=O) stretching; 1448, 1547 cm^{-1} (1535, 1603) probably caused by (C=C) stretching of the thiophene (furan) ring.

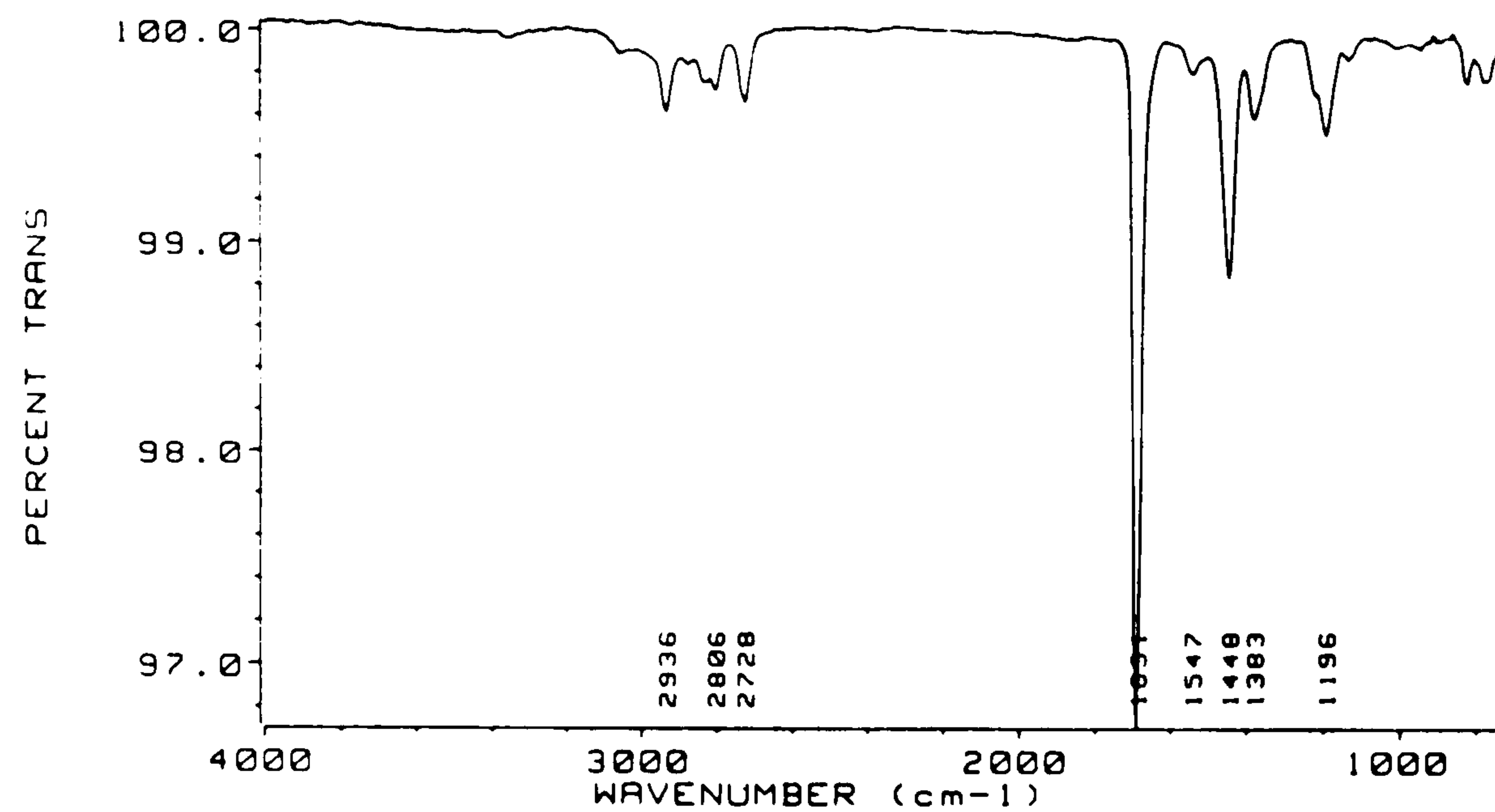
It has been suggested that formylthiophenes may be formed from the corresponding furfural and H_2S by a ring opening mechanism

Figure 2.3E: Infrared and mass spectra for compounds identified as a dimethylformylthiophene (compound 142) and a dimethylfurfural (compound 79 in Section 2.2)

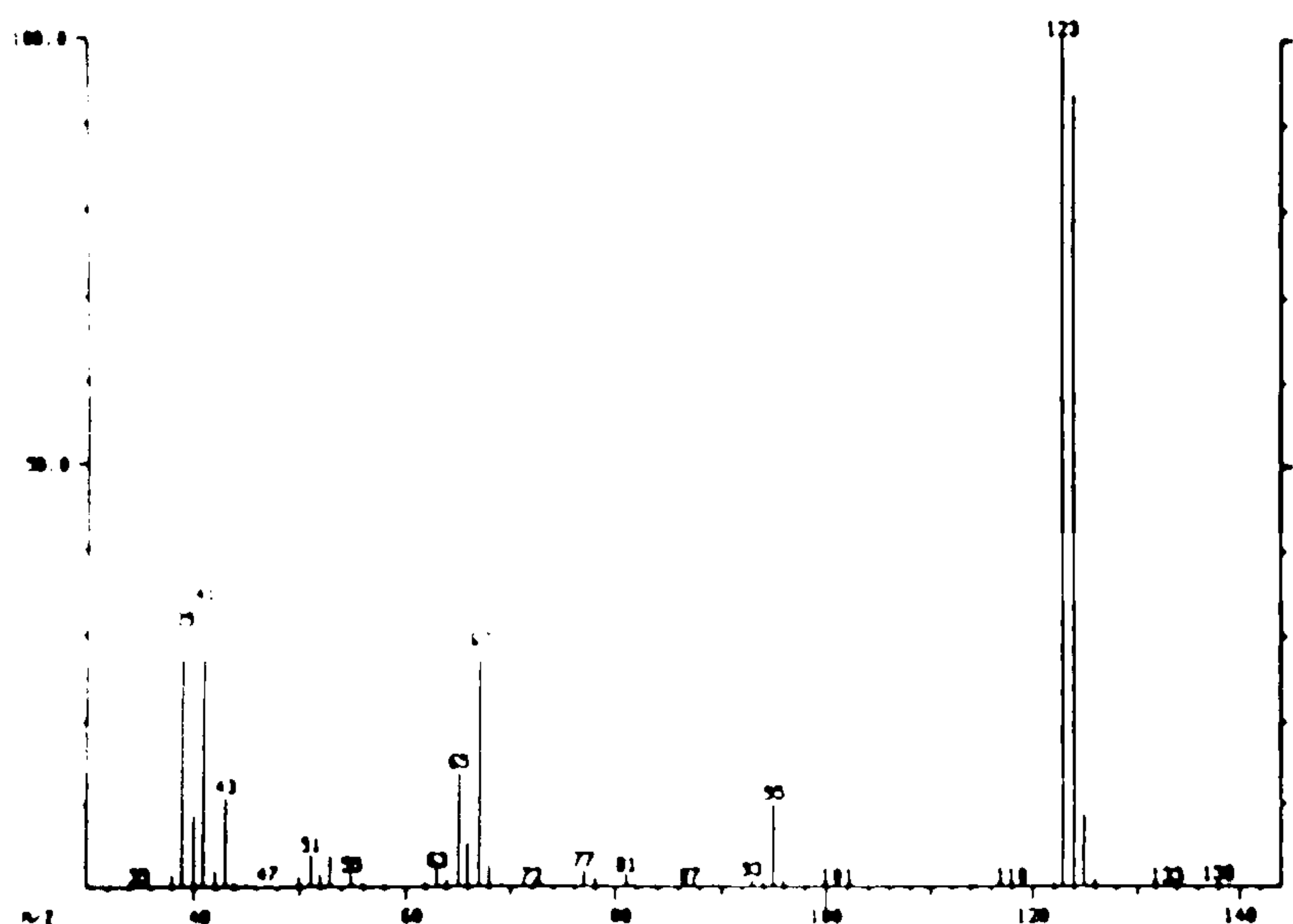
A dimethylformylthiophene (142)



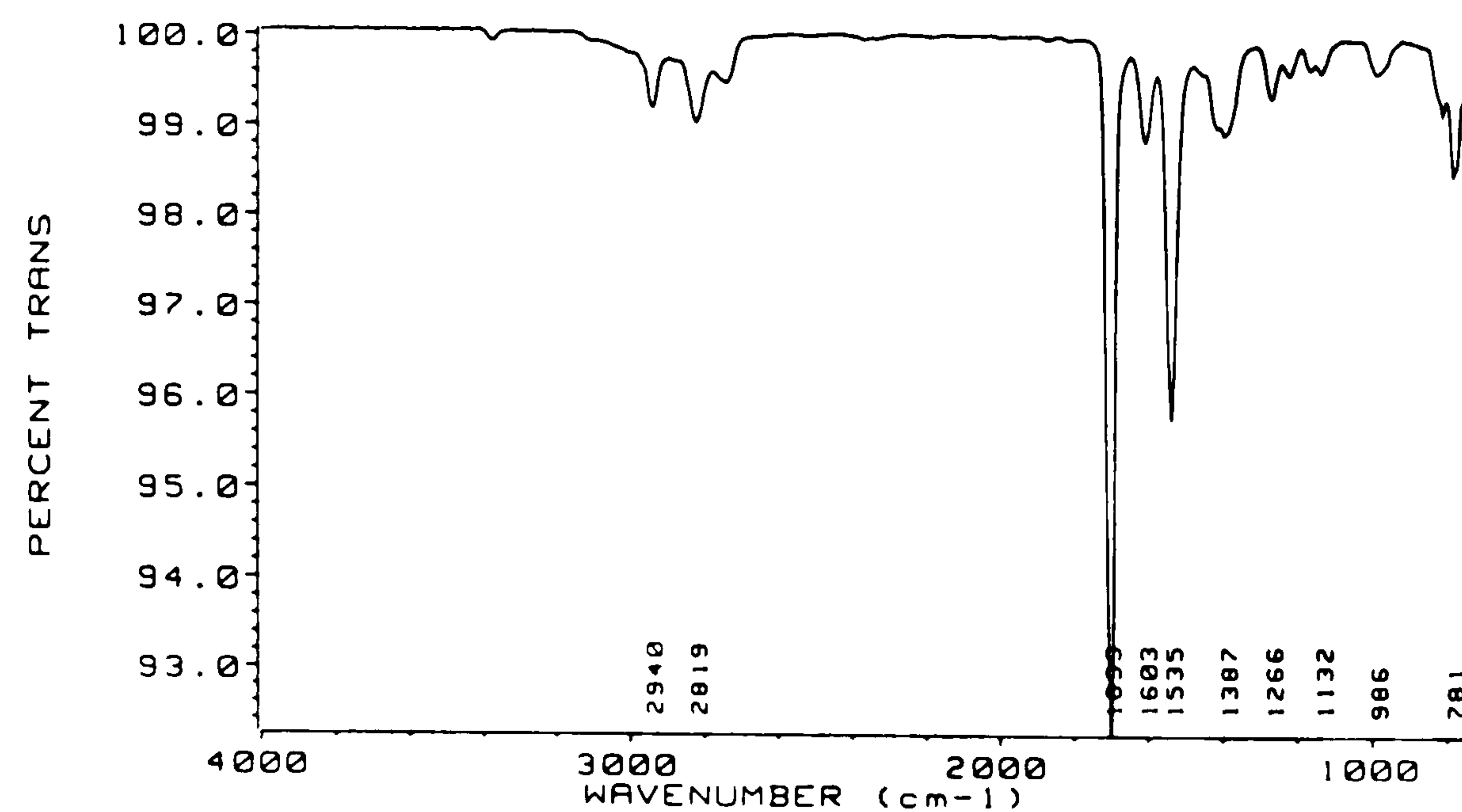
A dimethylformylthiophene (compound 142)



A dimethylfurfural (compound 79 in Sec. 2.2)



A dimethylfurfural (compound 79 in Section 2.2)



(Shibamoto 1977), or by the condensation of mercaptoacetaldehyde with α,β-unsaturated aldehydes (Mulders 1973); these mechanisms have been discussed in Section 1.1.1.3 and illustrated in Figure 1.1K.

Thiazoles

The mass spectra of many alkylthiazoles have been reported (Buttery *et al* 1973, Vitzthum and Werkhoff 1974a) and only two were detected for which neither the authentic material or a literature mass spectrum were available. For compound 51 the mass spectrum, with M^+ = 127 and the major loss of a methyl radical clearly suggested an ethylmethylthiazole. The loss of a fragment of mass 41 (CH_3CN) from the molecular ion indicated that the methyl group

Compound	MW	LRI	No.
Thiazole	85	1251	22
2-Methylthiazole	99	1242	20
2,5-Dimethylthiazole	113	1324	35
4,5-Dimethylthiazole	113	1377	47
Trimethylthiazole	127	1385	48
5-Ethyl-2-methylthiazole	127	1403	51
5-Ethyl-4-methylthiazole	127	1440	58
5-Ethyl-2,4-dimethylthiazole	141	1451	60
An ethylmethylthiazole	127	1489	67
2-Acetylthiazole	127	1639	97
2-Propionylthiazole	141	1721	113
2-Butanoylthiazole	155	1804	125
2-Methyl-(4 or 5)-propenylthiazole	139	1545	76
4,5-Dimethylisothiazole	113	1427	56

occupied the 2-position. 4-Ethyl-2-methylthiazole was ruled out as the mass spectral data and the LRI value did not agree with those of the authentic material; however, both would be consistent with an identity of 5-ethyl-2-methylthiazole.

4,5-Dimethylisothiazole (56) was identified by comparison with the mass spectrum of Heller and Milne (1978). The mass spectrum of compound 67 appeared to be that of an ethylmethylthiazole but did

not agree with data for the four isomers for which authentic material was available or with that predicted for the two which were not. The relatively late elution time of this compound may suggest that it is another isothiazole.

In addition to the alkyl substituted thiazoles, three acylthiazoles were generated in the cysteine + ribose system. An identity of 2-propionylthiazole was suggested for compound 113, by comparison of the infrared spectrum with that of 2-acetylthiazole: the spectra showed good agreement for all absorption bands down to 1300 cm^{-1} except that the intensity of the saturated (C-H) absorption was somewhat increased (Fig. 2.3F). This identity was supported by the mass spectrum which was analogous to that cited for 2-propionyl-4-methylthiazole by Stoll *et al* (1967). Likewise, the mass spectrum of compound 125 was consistent with an identity of 2-butanoylthiazole. Mulders (1973) has also identified these three acylthiazoles in the volatiles from a model reaction mixture containing cysteine and ribose and suggested a possible mechanism for their formation by the reaction of cysteine with α-dicarbonyls derived from the sugar.

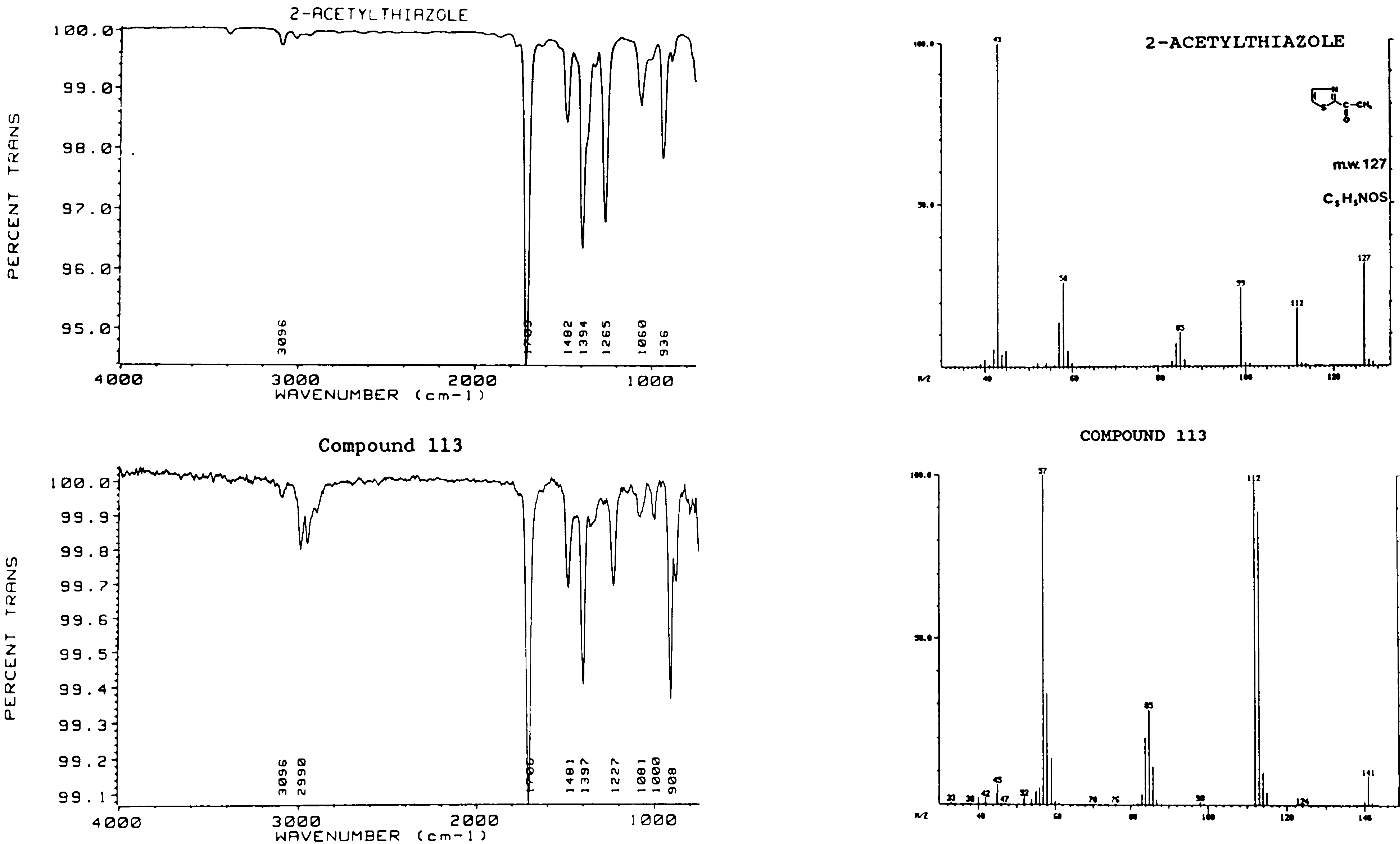
Thiazole itself, and most of the alkylthiazoles, have been found among the volatile components of coffee, cooked meats (in some cases) and other foods (van Straten and Maarse 1988). However, while 2-acetylthiazole has been reported in the volatiles from many foods, including beef and pork, no reference has been found to the occurrence of 2-propionyl- and 2-butanoylthiazoles in foods.

3-Methyl-1,2,4-trithiane and cis/trans-3,5-dimethyl-1,2,4-trithiolanes

Compound	MW	LRI	No.
cis-3,5-dimethyl-1,2,4-trithiolane	152	1586	87
trans-3,5-dimethyl-1,2,4-trithiolane	152	1607	90
3-methyl-1,2,4-trithiane	152	1857	133

Several compounds containing three sulphur atoms were identified; these included both the *cis* and the *trans* 3,5-dimethyl-1,2,4-trithiolanes (87, 90) together with the isomeric 3-methyl-1,2,4-

Figure 2.3F: Infrared and mass spectra for authentic 2-acetylthiazole and compound 113 (suggested identity : 2-propionylthiazole).



trithiane (133), which have all previously been identified in cysteine/cystine model systems with or without ribose (Mulders 1973; Shu *et al* 1985a,b). The compound, 3,5-dimethyl-1,2,4-trithiolane has also been detected in the volatiles from most cooked meats (van Straten and Maarse 1988), and may be formed from the cysteine breakdown products, ethanal and H_2S (Schutte 1974).

It has been established that *cis*-3,5-dialkyl-1,2,4-trithiolanes elute from polar GC columns before the corresponding *trans* isomers (Tjan *et al* 1972; Cuer *et al* 1979), and this relationship has been applied when assigning identities to the above trithiolanes and also to similar stereoisomers of the disubstituted thiophenones, dithiolanes and dithianones reported herein.

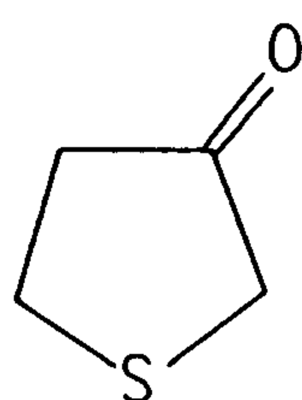
Thiophenones

Compound	MW	LRI	No.
Dihydro-3(2H)-thiophenone	102	1556	79
Dihydro-2-methyl-3(2H)-thiophenone	116	1522	74
Dihydro-4(5)-methyl-3(2H)-thiophenone	116	1528	75
cis-Dihydro-2,(4 or 5)-dimethyl-3(2H)-thiophenone	130	1478	65
trans-Dihydro-2,(4 or 5)-dimethyl-3(2H)-thiophenone	130	1482	66
Dihydro-2(5)-ethyl-3(2H)-thiophenone	130	1598	88
An ethyl-3(2H)-thiophenone	128	1790	123

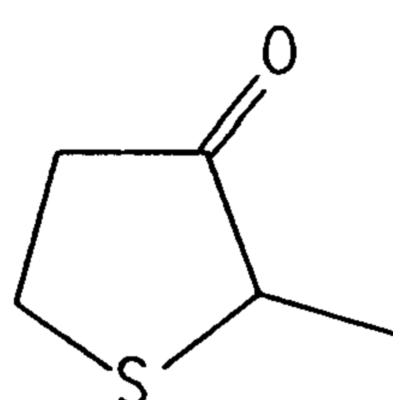
An interesting series of six dihydro-3(2H)-thiophenones (tetrahydrothiophen-3-ones) were detected (Fig. 2.3G). Authentic material was available for only two of these: dihydro-3(2H)-thiophenone (79) and its 2-methyl derivative (74). However, four other compounds were easily recognised as belonging to this class from the characteristic pattern of their mass spectra.

The three fragmentation pathways (Fig. 2.3H) deduced for the two fully authenticated members of this class may also explain the major ions in the other dihydro-3(2H)-thiophenones detected. Path (a) appears to be the dominant route of fragmentation, yielding ions m/z 74 and 46 for dihydro-3(2H)-thiophenone and m/z 88 and 60 for dihydro-2-methyl-3(2H)-thiophenone. The atomic composition of these fragments has been confirmed for dihydro-2-methyl-3(2H)-

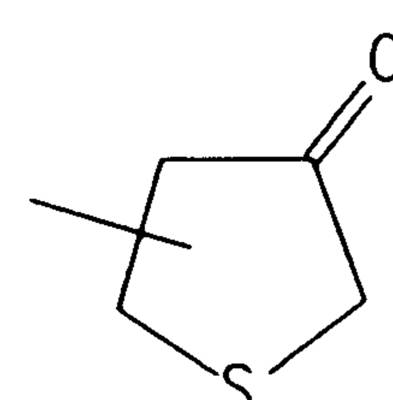
Figure 2.3G: Some 3(2H)-thiophenones formed in the reaction between cysteine and ribose



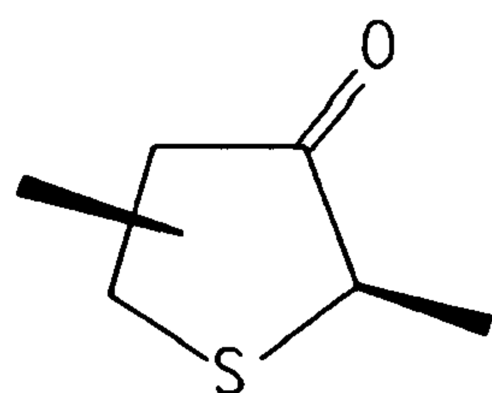
dihydro-3(2H)-thiophenone
(79)



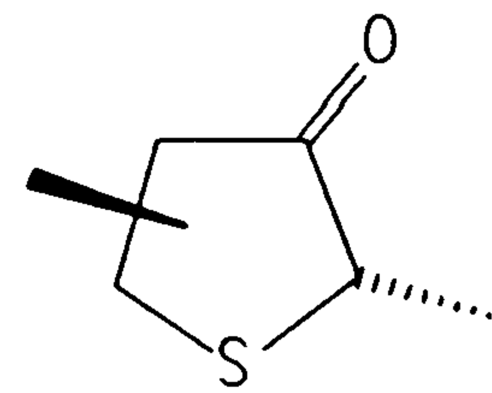
dihydro-2-methyl-
3(2H)-thiophenone (74)



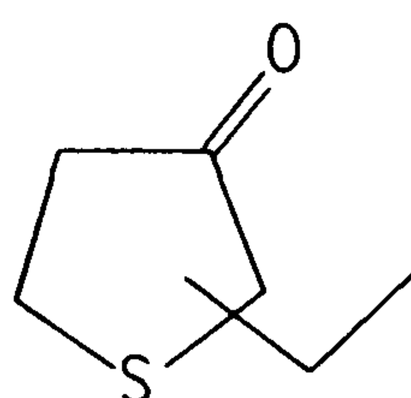
dihydro-4(or 5)-methyl-
3(2H)-thiophenone (75)



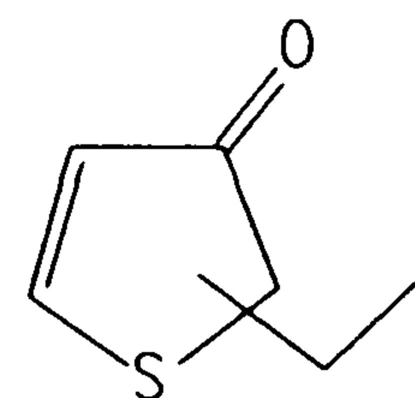
cis-dihydro-2,(4 or 5)-dimethyl
3(2H)-thiophenone (65)



trans-dihydro-2,(4 or 5)-dimethyl
3(2H)-thiophenone (66)



dihydro-2(or 5)-ethyl-
3(2H)-thiophenone (88)



an ethyl-3(2H)-thiophenone (123)

Figure 2.3H: Suggested fragmentation pathways for dihydro-3(2H)-thiophenones

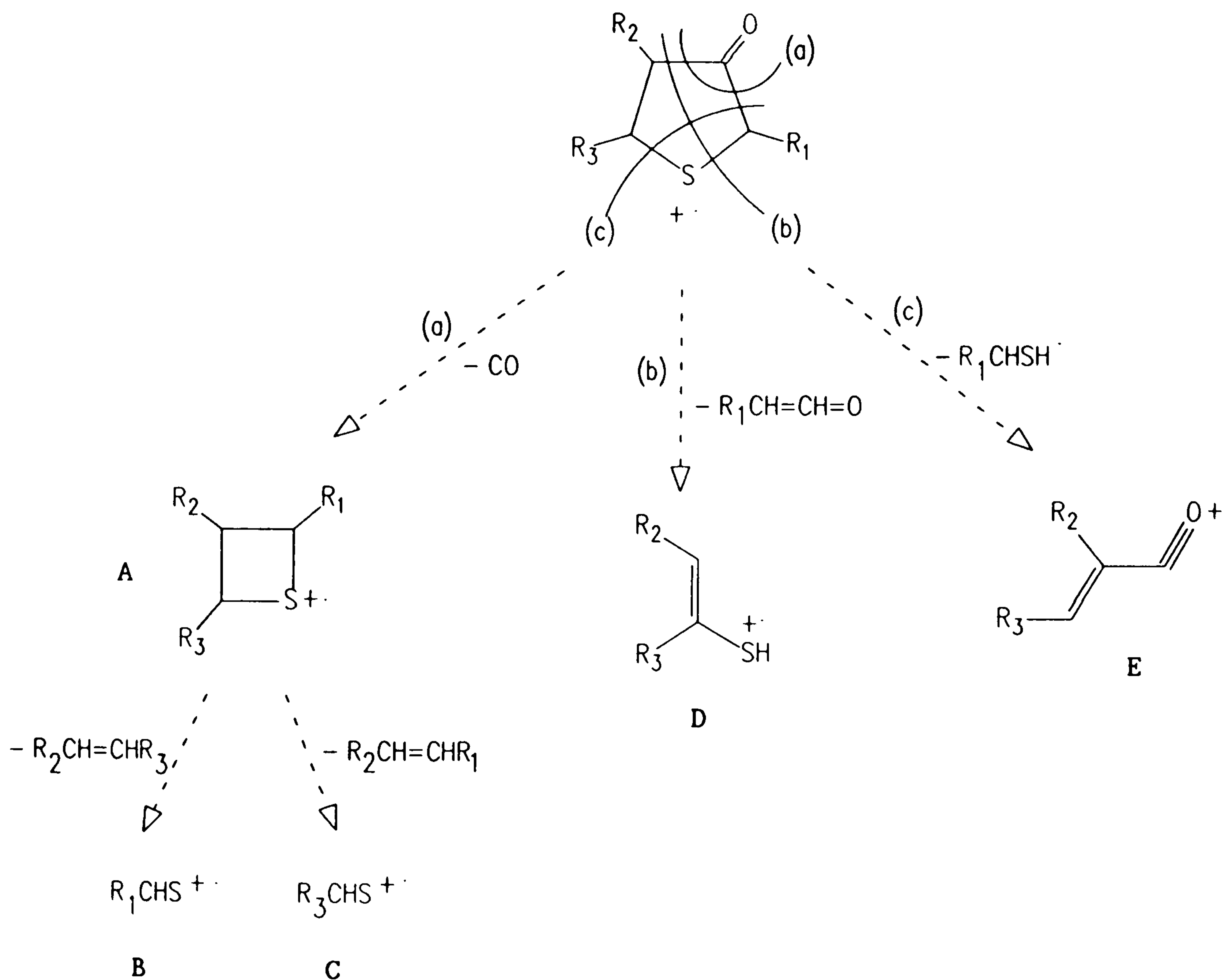


Table 2.3b: Possible fragment ions from dihydro-3(2H)-thiophenones, by pathways illustrated in Figure 2.3H.

Dihydro-alkyl-3(2H)-thiophenone alkyl =	R_1	R_2	R_3	M^+	Predicted fragments[a] A	B	C	D	E	Matches data for compound number:
--	H	H	H	102	74	46	46	60	55	79
-2-methyl-	Me	H	H	116	88	60	46	60	55	74
-4-methyl-	H	Me	H	116	88	46	46	74	69	75
-5-methyl-	H	H	Me	116	88	46	60	74	69	75
-2,4-dimethyl-	Me	Me	H	130	102	60	46	74	69	65,66
-2,5-dimethyl-	Me	H	Me	130	102	60	60	74	69	65,66
-4.5-dimethyl-	H	Me	Me	130	102	46	60	88	83	-
-2-ethyl-	Et	H	H	130	102	74	46	60	55	88
-4-ethyl-	H	Et	H	130	102	46	46	88	83	-
-5-ethyl-	H	H	Et	130	102	46	74	88	83	88

a Predicted fragments were compared with ions of observed mass spectra with appropriate molecular ion

thiophenone and also for compounds 65 and 66. Fragmentation by such a route has been suggested for dihydro-3(2H)-furanones (Porter 1985). The base peak is given by fragment A or B; in the case of dihydro-2-methyl-3(2H)-thiophenone, where these fragments are not identical, the loss of ethylene to give m/z 60 is favoured, almost to the exclusion of m/z 46. Either the larger S-containing fragment is more stable or cleavage of the S-C bond occurs before the 4-membered ring structure is formed, favouring the loss of an alkene derived from the 3 and 4 positions of the thiophenone.

Table 2.3b shows the predicted ions for all possible methyl, dimethyl and ethyl substituted dihydro-3(2H)-thiophenones, with the compound numbers of the detected dihydro-3(2H)-thiophenones assigned to their likely identities. As a number of these isomers give the same predicted fragments, the precise identities of the unknown dihydro-3(2H)-thiophenones cannot be ascertained from the mass spectral data alone.

The fragment ions observed for compound 75 were consistent with either the 4- or 5-methyl derivative, but the absence of any ion at m/z 60 may suggest that the dihydro-4-methyl-3(2H)-thiophenone is the more likely. The two dihydro-3(2H)-thiophenones (65, 66) with MW 130 eluted within 4 LRI units of each other and possessed identical mass spectra, consistent with an identity of either 2,4- or 2,5-dimethyl isomers. However, these two isomers would be expected to show some differences in their mass spectra and retention times; thus, compounds 65 and 66 were almost certainly the *trans*- and *cis*- versions of one of these isomers. The elution behaviour of these compounds (see Section 2.3.2.2) may favour an identity of 2,4-dimethyl-dihydro-3(2H)-thiophenone.

The infrared spectra of compounds 65, 66 and 88 compared well with reference spectra for dihydro-3(2H)-thiophenone and the 2-methyl derivative, albeit with more intense absorbances due to saturated C-H bonds; for compound 88, the close similarity of the region below 1600 cm^{-1} with that of dihydro-2-methyl-3(2H)-thiophenone suggests that the ethyl substituent is probably in the 2 position.

The mass spectrum of compound 123 had a very similar pattern to that of dihydro-(2 or 5)-ethyl-3(2H)-thiophenone but the m/z values for all ions were reduced by 2 mass units. The molecular

formula, C_6H_8OS also indicates an increased degree of unsaturation and this compound was tentatively identified as an ethyl-3(2H)-thiophenone.

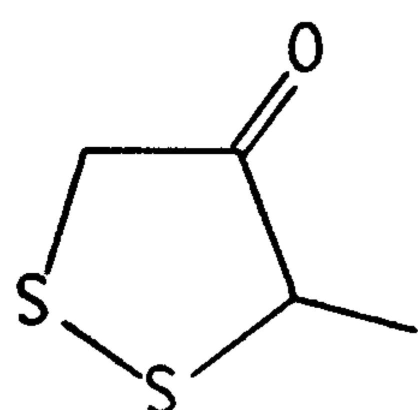
Dihydro-3(2H)-thiophenone and its 2-methyl derivative have been detected in the volatile compounds from cooked beef and various other foods and drinks (van Straten and Maarse 1988) and are known to have a garlic flavour and an acetylenic odour respectively (Fors 1983). Dihydro-3(2H)-thiophenones have also been reported as products of various model reactions involving the heating of sugars or their breakdown products with cysteine or H_2S (Kato *et al* 1973b; van den Ouweland and Peer 1975), or, in the case of dihydro-3(2H)-thiophenone, from cysteine alone (Shu *et al* 1985a). While some of these compounds may be formed directly from hydroxyfuranones and H_2S (van den Ouweland and Peer 1975), a mechanism similar to that suggested by Piloty and Baltes (1979) for 3(2H)-furanones, involving the condensation of two dicarbonyl compounds, might explain the formation of dihydro-3(2H)-thiophenones with more than five carbon atoms.

1,2-Dithiolan-4-ones and 1,2-dithian-4-ones

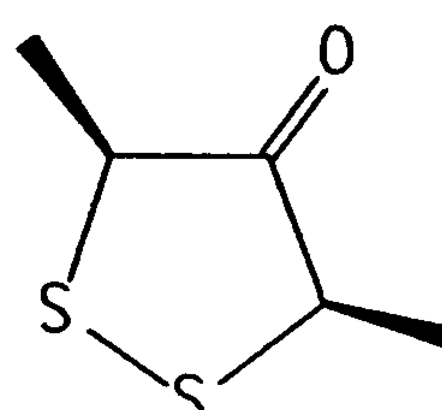
Compound	MW	LRI	No.
3-Methyl-1,2-dithiolan-4-one	134	1664	101
cis-3,5-Dimethyl-1,2-dithiolan-4-one	148	1574	83
trans-3,5-Dimethyl-1,2-dithiolan-4-one	148	1604	89
cis-3-Ethyl-5-methyl-1,2-dithiolan-4-one	162	1653	98
trans-3-Ethyl-5-methyl-1,2-dithiolan-4-one	162	1676	108
1,2-Dithian-4-one	134	1930	143
3-Methyl-1,2-dithian-4-one	148	1894	139
cis-3,(5 or 6)-Dimethyl-1,2-dithian-4-one	162	1847	132
trans-3,(5 or 6)-Dimethyl-1,2-dithian-4-one	162	1876	137

Nine components with the molecular formula, $C_nH_{2n-2}OS_2$ were identified as belonging to two compound classes containing either 5 or 6 membered rings with adjacent S atoms: 1,2-dithiolan-4-ones (83, 89, 98, 101, 108) or 1,2-dithian-4-ones (132, 137, 139, 143). Proposed structures for these compounds are shown in Figure 2.3I). Accurate mass data were available for compounds 89, 139 and 143; for the remaining compounds the abundance of the $M+2^+$ ion showed

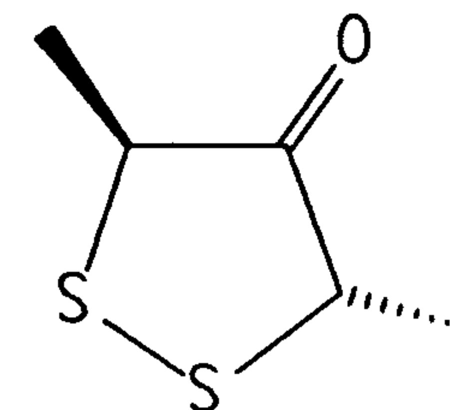
Figure 2.31: Prabable structures of 1,2-dithiolan-4-ones and 1,2-dithian-4-ones from the reaction between cysteine and ribose



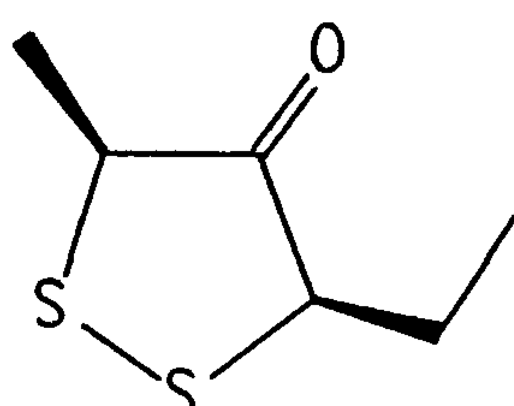
3-methyl-1,2-dithiolan-4-one (101)



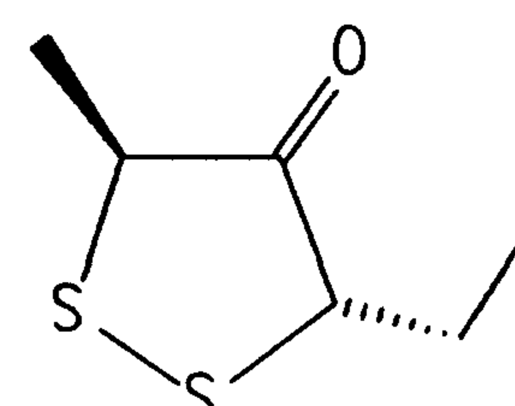
cis-3,5-dimethyl-1,2-dithiolan-4-one (83)



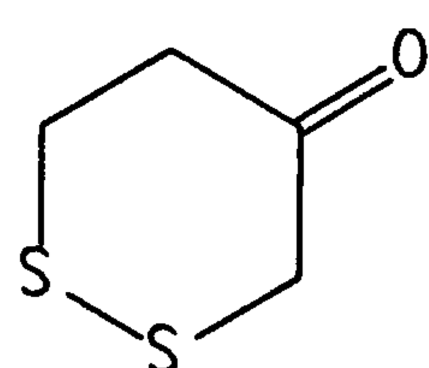
trans-3,5-dimethyl-1,2-dithiolan-4-one (89)



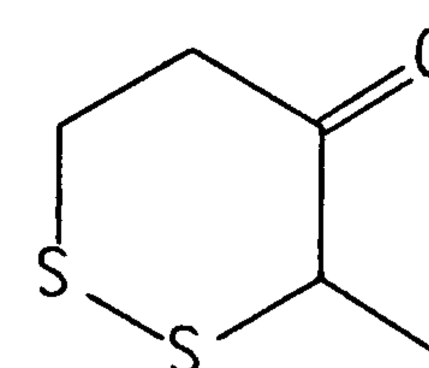
cis-3-ethyl-5-methyl-1,2-dithiolan-4-one (98)



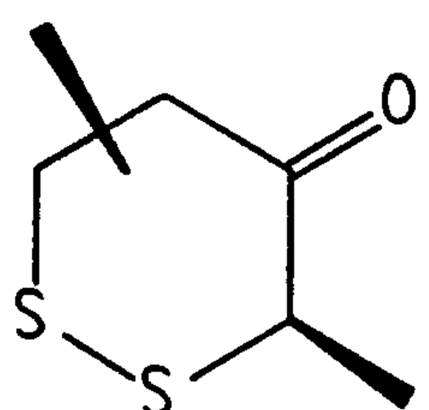
trans-3-ethyl-5-methyl-1,2-dithiolan-4-one (108)



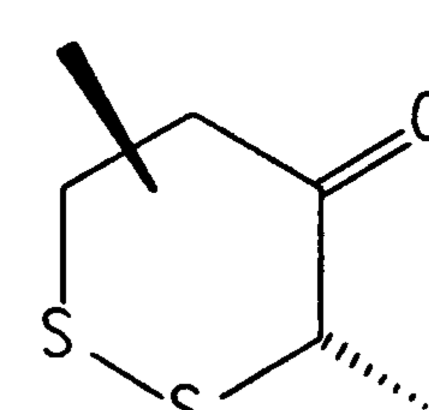
1,2-dithian-4-one (143)



3-methyl-1,2-dithian-4-one (139)



cis-3,(5 or 6)-dimethyl 1,2-dithian-4-one (132)



trans-3,(5 or 6)-dimethyl 1,2-dithian-4-one (137)

the presence of two S atoms and the overall pattern of the mass spectra indicated that they belonged to these compound classes. The mass spectral fragmentation pathways of these two groups were similar with major ions resulting from the molecular ion and from the cleavage of the ring across the S-S bond to yield a fragment, $RCHS^{+}$. However, members of the two classes were readily distinguished by the fact that the dithianones eluted approximately 200 LRI units after the isomeric dithiolanones.

Probable fragmentation pathways were deduced for the members of these classes for which literature mass spectra corroborated the suggested identity (compounds 83, 89, 101, 139). Tentative identities for the remaining compounds were deduced by comparison of the fragment ions expected from possible isomers of the unknown compounds with those actually observed; see Figure 2.3J and Table 2.3c for the dithiolanones and Figure 2.3K and Table 2.3d for the dithianones.

Only two of the detected 1,2-dithiolan-4-ones (98 and 108) had not been reported previously and these were readily identified from the mass spectral data as the *cis*- and *trans*-3-ethyl-5-methyl-1,2-dithiolan-4-ones; the predicted fragment ions accorded well with the recorded mass spectra (Figure 2.3J; Table 2.3c).

While 1,2-dithian-4-one (143) itself could be readily identified in a similar manner, the precise substitution pattern of compounds 132 and 137 was unclear. The close similarity of their mass spectra indicated that they were the *cis* and *trans* forms of the same isomer. The absence of m/z 46 and 106 (from fragments E and I) indicated that there must be substituents in both the 3-position and either the 5- or 6-position (Fig. 2.3K, Table 2.3d). Thus, a 3,5-dimethyl- or 3,6-dimethyl-1,2-dithian-4-one was indicated. However, as the predicted ions from these isomers were identical it was not possible to distinguish them by this method. The elution behaviour of these compounds (see Section 2.3.2.2) may provide some tentative evidence for the *cis*- and *trans*-3,5-dimethyl-1,2-dithian-4-one.

Infrared spectra were recorded for two of these compounds, 1,2-dithian-4-one and 3-methyl-1,2-dithian-4-one. The latter showed the same absorption bands as reported by Hartman *et al* (1984b). The spectra of these two compounds were very similar to those for

Figure 2.3J: Suggested fragmentation pathways for 1,2-dithiolan-4-ones

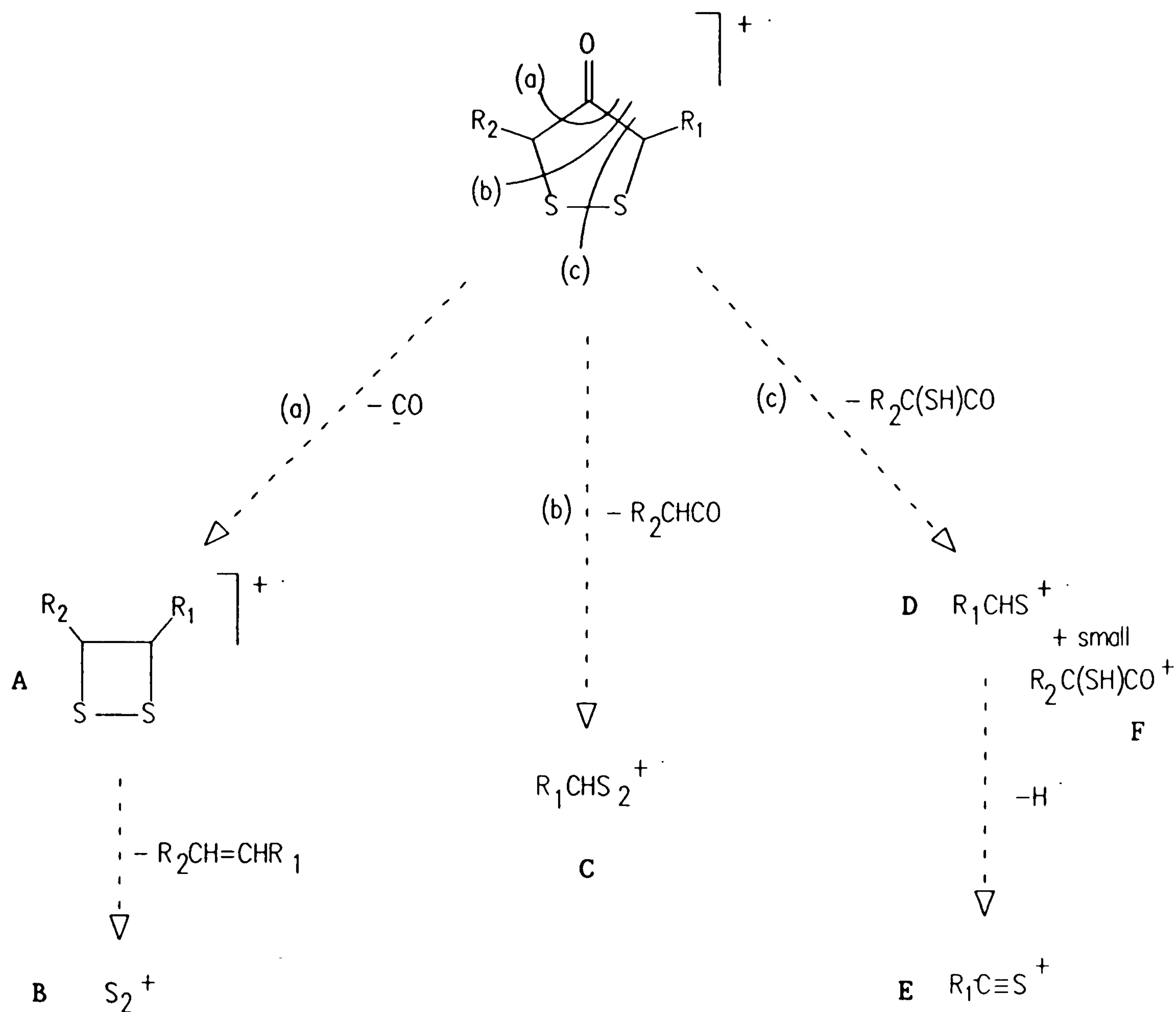


Table 2.3c: Possible fragment ions from 1,2-dithiolan-4-ones, by pathways illustrated in Figure 2.3J.

-1,2-dithiolan-4-one		R_1	R_2	M^+	Predicted fragments[a] A	B	C	D	E	F	Matches data for compounds number:
3-methyl-		Me	H	134	106	64	92	60	59	74	101
	or	H	Me				78	46	45	88	
3,5-dimethyl-		Me	Me	148	120	64	92	60	59	88	83,89
3-ethyl-		Et	H	148	120	64	106	74	73	74	-
	or	H	Et				78	46	45	102	
3-ethyl-5-methyl-		Et	Me	162	134	64	106	74	73	88	98,108
	or	Me	Et				92	60	59	102	
3-propyl-		Pro	H	162	134	64	120	88	82	74	-
	or	H	Pro				78	46	45	116	

a Predicted fragments were compared with ions of observed mass spectra with appropriate molecular ion

Figure 2.3K: Suggested fragmentation pathways for 1,2-dithian-4-ones

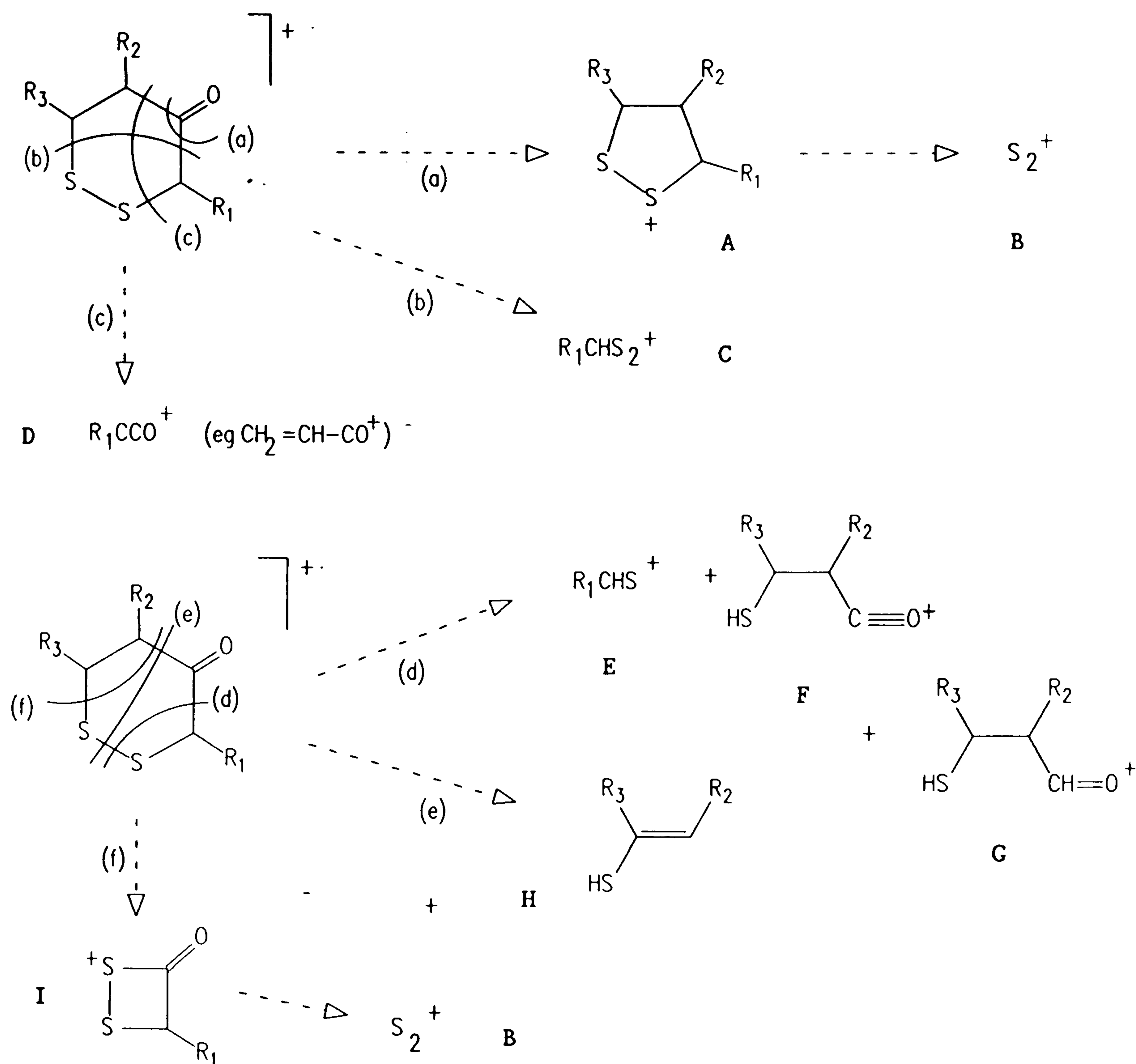


Table 2.3d: Possible fragment ions from 1,2-dithian-4-ones, by pathways illustrated in Figure 2.3K.

-1,2-dithian-4-one	R_1	R_2	R_3	M^+	Predicted fragments[a] A	B	C	D	E	F	G	H	I	Matches data for compounds number:
1,2-dithian-4-one	H	H	H	134	106	64	78	-	46	89	88	60	106	143
3-methyl-	Me	H	H	148	120	64	92	55	60	89	88	60	120	139
5-methyl-	H	Me	H	148	120	64	78	-	46	103	102	74	106	-
6-methyl-	H	H	Me	148	120	64	78	-	46	103	102	74	106	-
3,5-dimethyl-	Me	Me	H	162	134	64	92	55	60	103	102	74	120	132,137
3,6-dimethyl-	Me	H	Me	162	134	64	92	55	60	103	102	74	120	132,137
5,6-dimethyl-	H	Me	Me	162	134	64	78	-	46	117	116	88	106	-
3-ethyl-	Et	H	H	162	134	64	106	69	74	89	88	60	134	-
5-ethyl-	H	Et	H	162	134	64	78	-	46	117	116	88	106	-
6-ethyl-	H	H	Et	162	134	64	78	-	46	117	116	88	106	-

of observed mass spectra with appropriate molecular ion.

dihydro-3(2H)-thiophenone and dihydro-2-methyl-3(2H)-thiophenone, which differed only by the absence of one S atom. The (C=O) stretching band of 1,2-dithian-4-one and its 3-methyl derivative (1730, 1725 cm^{-1} respectively) occurred at a slightly lower energy than the same absorption in the related dihydro-3(2H)-thiophenones (1758, 1752 cm^{-1}), probably due to the lesser degree of strain in the 6-membered ring.

Only two of these compounds have been previously reported as the products of model reactions; 3-methyl-1,2-dithiolan-4-one (101) is a product of the thermal degradation of cysteine (Shu *et al* 1985a), while 3-methyl-1,2-dithian-4-one (137) was formed on heating a meat flavour model system containing glutamate, ascorbic acid, thiamine and cystine or from thiamine alone (Hartman *et al* 1984a,b). Hartman *et al* (1984b) suggested that this compound may be formed by a similar mechanism to the dihydro-3(2H)-thiophenones as the structures differ by only one sulphur atom.

Bicyclic compounds

Compound	MW	LRI	No.
2,3-Dihydro-6-methylthieno[2,3c]furan	140	1728	115
Thieno[2,3b]thiophene	140	1865	134
Thieno[3,2b]thiophene	140	1874	136
Thieno[3,4b]thiophene	140	1976	147
A methylthienothiophene	154	1918	141
A methylthienothiophene	154	1940	144
A dihydrothienothiophene	142	1910	140
A dihydrothienothiophene	142	1962	146
A dihydrothienothiophene	142	2022	149
A methyldihydrothienothiophene	156	1999	148
A methyldihydrothienothiophene	156	2038	150
A methyldihydrothienothiophene	156	2103	152
A dimethyldihydrothienothiophene	170	2077	151

2,3-Dihydro-6-methylthieno[2,3c]furan (kahweofuran) (115), an important constituent of coffee volatiles, was identified by comparison of the mass and infrared spectra with both the abbreviated data reported by Buchi *et al* (1971) and with those given by the authentic sample.

Three thienothiophenes (**134**, **136**, **147**) were found, together with two compounds (**141**, **144**) tentatively identified as methylthienothiophenes. High resolution mass spectrometry gave a molecular formula for these two compounds of $C_7H_6S_2$; the degree of unsaturation indicated a highly cyclic structure, while the mass spectra contained many of the same ions as the unsubstituted thienothiophenes. The much higher abundance of the $M\text{-}1^+$ ion could be explained by the loss of H from the methyl substituent, followed by formation of a stable 6-membered thiopyrilium ring structure, not possible in the unsubstituted thienothiophenes. Thieno[2,3b]thiophene and thieno[3,2b]thiophene have been reported in the volatile components of coffee (Stoll *et al* 1967).

Only tentative suggestions could be made concerning the identities of another series of compounds also containing two sulphur atoms. The molecular weights of these components were 142 (compounds **140**, **146**, **149**), 156 (compounds **148**, **150**, **152**) and 170 (compound **151**), two mass units higher than thienothiophenes. Like the thienothiophenes, the mass spectra were dominated by the M^+ and $M\text{-}1^+$ ions, albeit with an additional large m/z 97 in the case of those compounds with MW = 142. The general molecular formula, $C_nH_{2n-6}S_2$ again indicated a high degree of unsaturation. However, the infra red spectra of these compounds showed very little absorption due to unsaturated (C-H) stretching but had relatively large absorbances due to saturated (C-H); indeed the spectra of compounds **149**, **151** and **152** had many bands in common with 2-propylthiophene. Thus, it seemed probable that the degrees of unsaturation were in part due to a highly cyclic structure and that these compounds were the bicyclic dihydrothienothiophenes.

Long-chained heterocyclic compounds and the alkanethiols

The compounds listed in this section were not generated by the basic Maillard reaction between cysteine and ribose; their formation was specific to the presence of lipid.

The 2-alkylfurans are known products of lipid oxidation and the 2-alkylthiophenes could be derived from the action of H_2S on the corresponding furans at high temperature, as suggested by Vernin and Parkanyi (1982). Alternatively, they could have arisen from

Compound	MW	LRI	No.
2-Pentylpyridine	149	1580	85
2-Methyl-5-propylthiophene	140	1306	32
2-Butylthiophene	140	1338	41
2-Pentylthiophene	154	1438	57
2-Hexylthiophene	168	1547	77
2-Heptylthiophene	182	1655	99
2-Pentylthiapyran	168	1661	100
cis or *trans*-2-(1-Hexenyl)-thiophene	166	1695	110
cis or *trans*-2-(1-Hexenyl)-thiophene	166	1724	114
2-(1-Heptenyl)-thiophene	180	1835	130
1-Heptanethiol	132	1231	18
1-Octanethiol	146	1335	40

the reaction of H_2S with dienals. 2-Pentylpyridine (**85**) is thought to arise from the reaction of NH_3, from the Strecker degradation of cysteine, with 2,4-decadienal (Buttery *et al* 1977).

Eluting very close to 2-heptylthiophene (**99**) was a compound (**100**) with an almost identical mass spectrum and the same molecular formula as 2-hexylthiophene, albeit with a smaller molecular ion. Both 3-hexylthiophene and pentylthiapyran might be expected to give mass spectra similar to 2-hexylthiophene as the formation of the 6-membered ring, $C_5H_5S^+$ (m/z 97) would be favoured in all three cases. The compound showed a large GC peak, and an identity of 2-pentylthiapyran is favoured because the formation of large amounts of 3-hexylthiophene cannot be predicted from straight chain fatty acids.

Suggested routes of formation for 2-pentylpyridine, 2-hexylthiophene and 2-pentylthiapyran from 2,4-decadienal are shown in Figure 2.3L.

Two compounds (**110**, **114**), with identical mass spectra (MW = 166), together with the related compound **130** (MW = 180), were tentatively identified as *cis*- and *trans*-2-(1-hexenyl)thiophene and a 2-(1-heptenyl)thiophene. The mass spectral fragmentation patterns (see Figure 2.3M) were consistent with these structures

Figure 2.3L: Suggested routes of formation for 2-pentylpyridine, 2-hexylthiophene and 2-pentylthiapyran from 2,4-decadienal

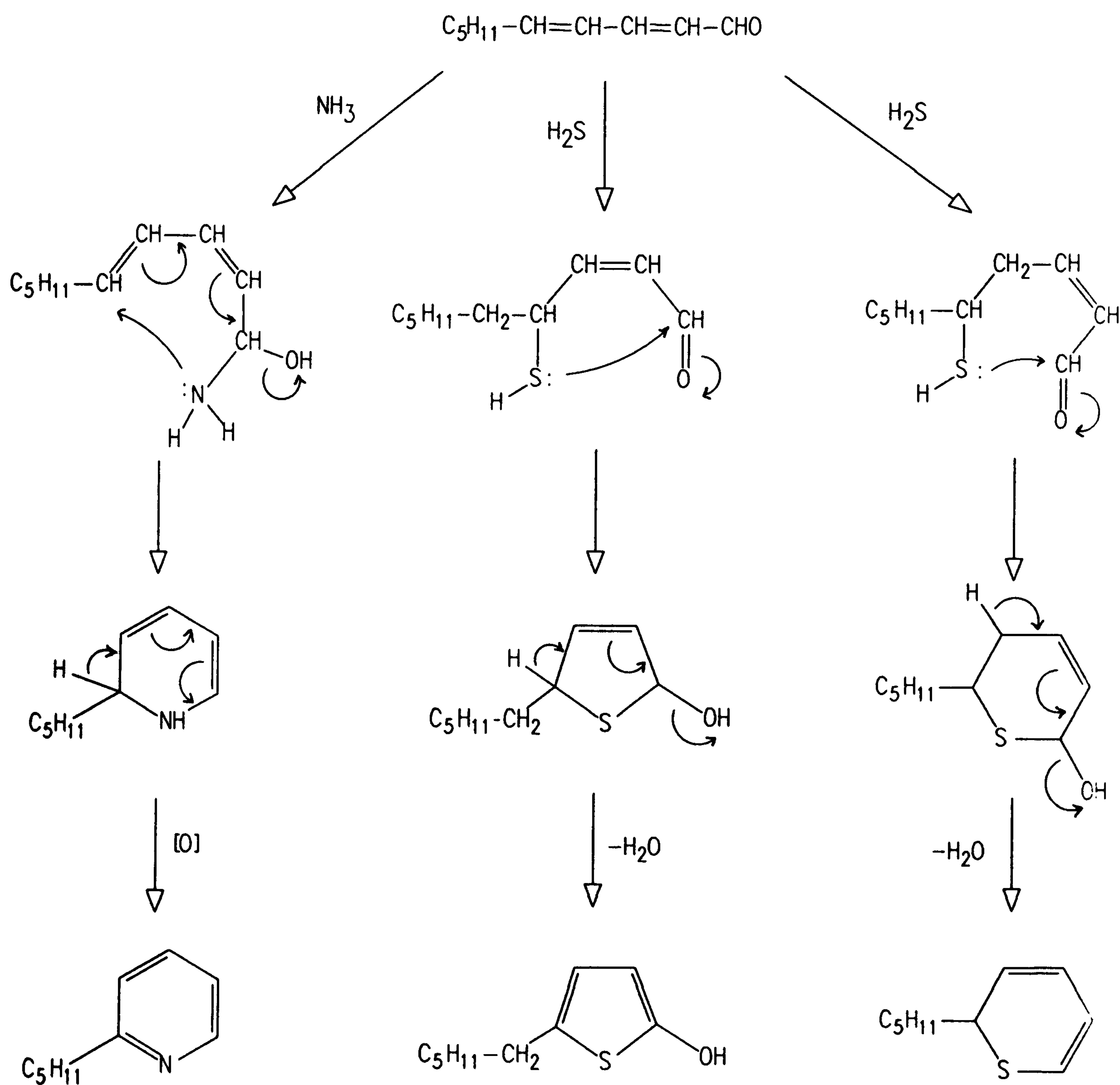
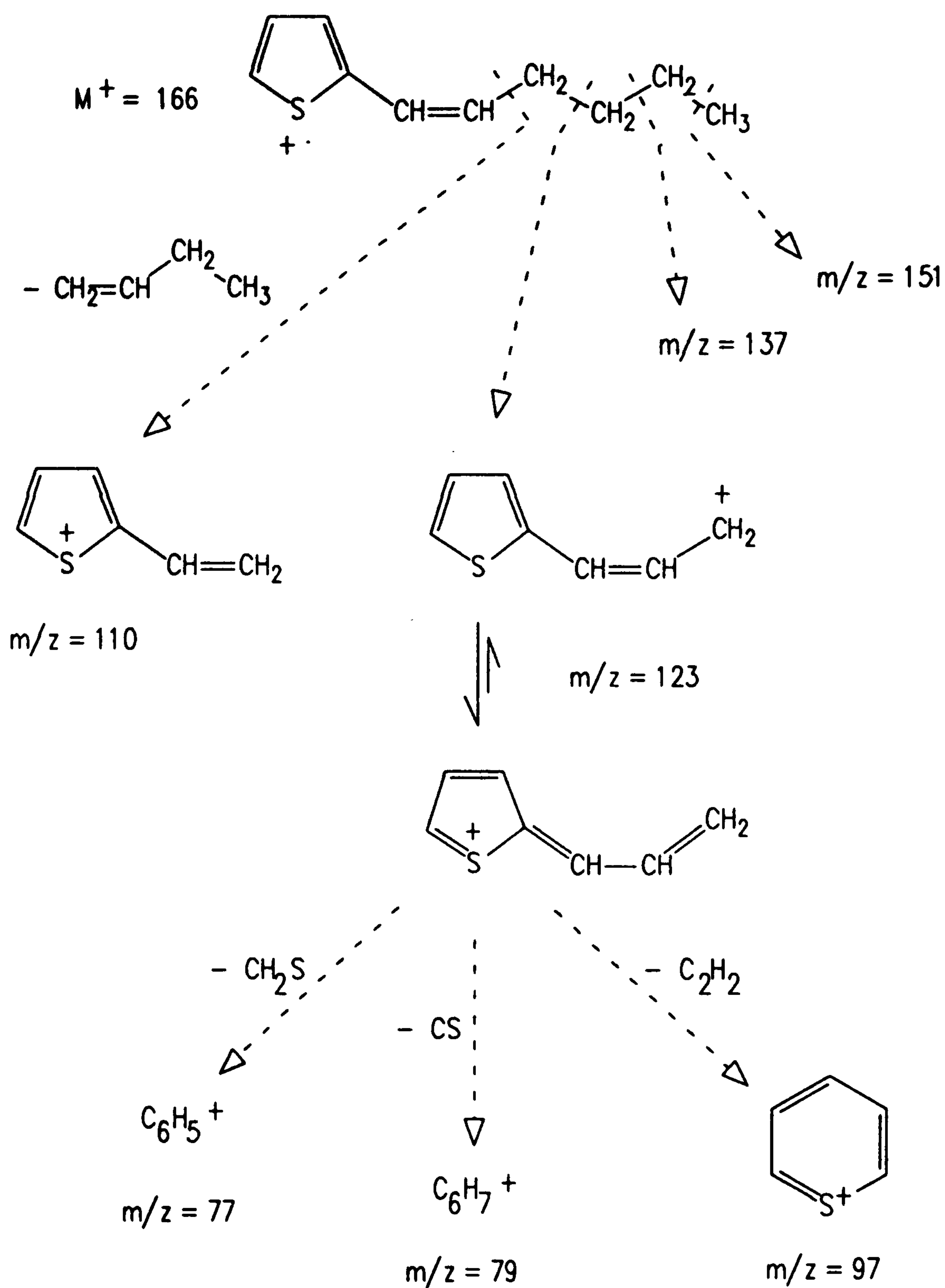
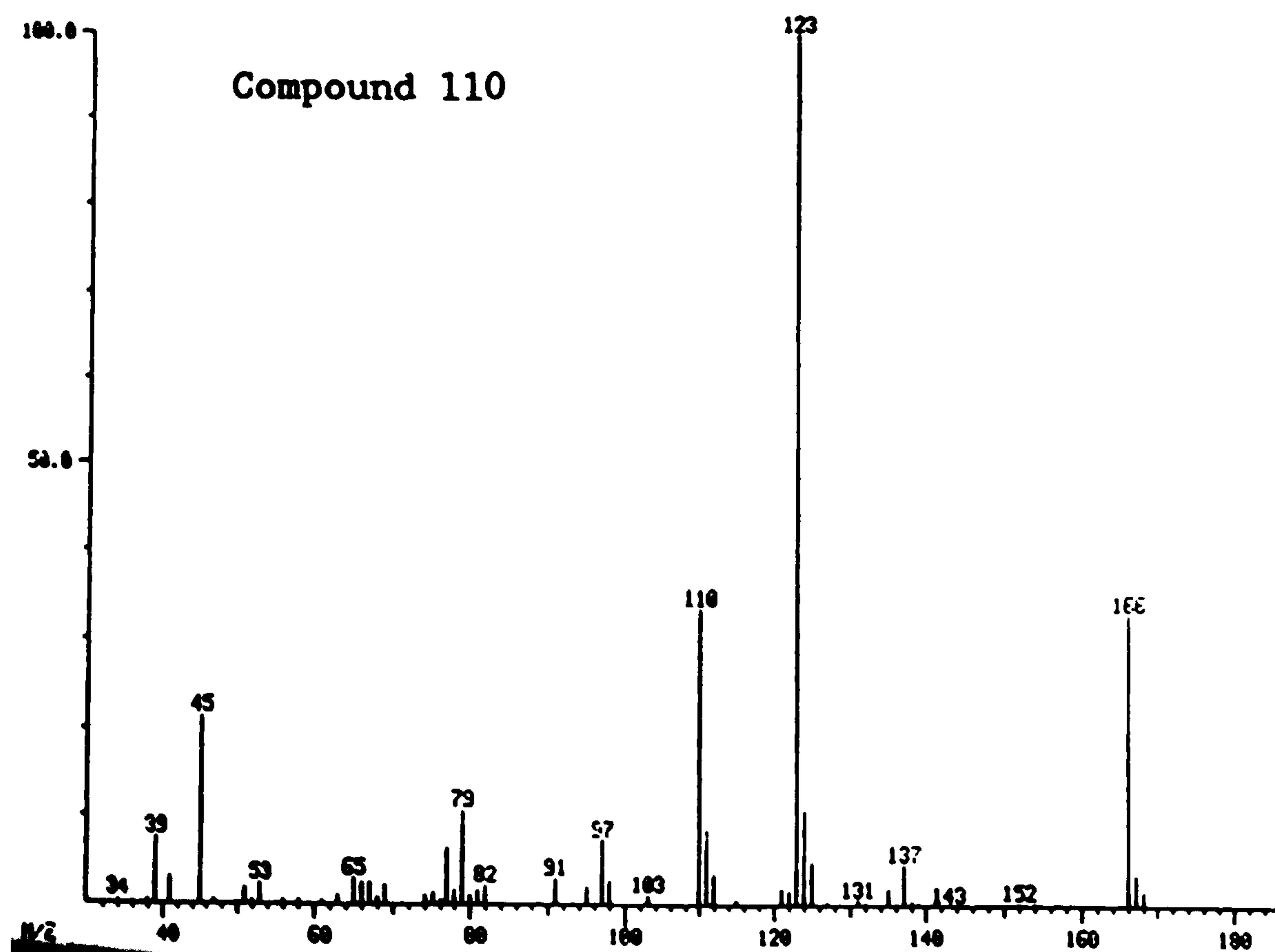


Figure 2.3M: Suggested mass spectral fragmentation pathway for *cis* and *trans*-2-(1-hexenyl)thiophene (110, 114)





and were analogous to that of 2-(1-pentenyl)-furan (Ho *et al* 1978). These compounds have not previously been reported in foods (van Straten and Maarse 1988) or model systems.

The two alkanethiols, 1-heptanethiol (**18**) and 1-octanethiol (**40**) were also formed only in the presence of phospholipid; they have both been detected among the volatile components obtained from beef (van Straten and Maarse 1988) and probably arise from the action of H_2S on lipid breakdown products, such as alcohols or aldehydes.

2.3.2 Elution behaviour of dithiolanones, dithianones and dihydro-3(2H)-thiophenones.

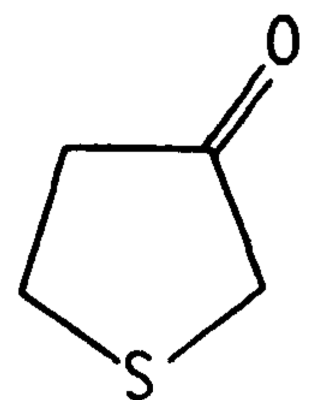
One unusual feature shared by the dihydro-3(2H)-thiophenones, the 1,2-dithiolan-4-ones and the 1,2-dithian-4-ones was that the introduction of additional methyl substituents reduced the retention time on the polar-phased column; i.e. the unsubstituted compound eluted after the monomethyl derivative and the dimethyl derivative eluted earliest. A similar effect was noted with 2-acetyl-3-methylthiophene (**119**) and 3-acetylmethylthiophene (**117**) both of which eluted before the corresponding acetylthiophenes (**121**, **120**). In contrast, the 4-methyl and 5-methyl-substituted 2-acetylthiophenes (authentic compounds) eluted 100 LRI units later (see Fig. 2.3N).

A possible explanation for this behaviour is that some weak intramolecular interaction, or H-bonding, occurs between the keto group in each case and the hydrogen of an adjacent methyl group, which reduces the polarity of the molecule. Thus, the dithiolanones with a methyl group each side of the keto group (**83**, **89**) eluted before that with only one adjacent methyl (**101**). Similarly, the dithianone with a methyl group in the 3 position (**139**) eluted earlier than 1,2-dithian-4-one itself (**143**) while the fact that the dimethyl-1,2-dithian-4-ones (**132**, **137**) eluted earliest may suggest that the substitution pattern is 3,5- rather than 3,6-dimethyl.

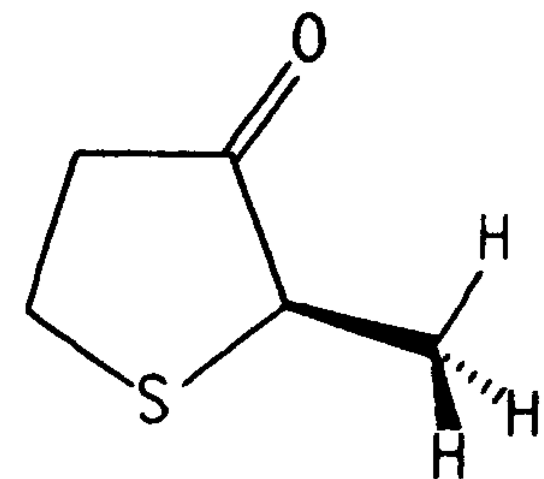
The mass spectral data for the dihydro-3(2H)-thiophenones do not permit the precise differentiation of isomers. However, both the known 2-methyl substituted compound (**74**) and its isomer (**75**)

Figure 2.3N: Structures of compounds showing unusual elution behaviour

Dihydro-3(2H)-thiophenones

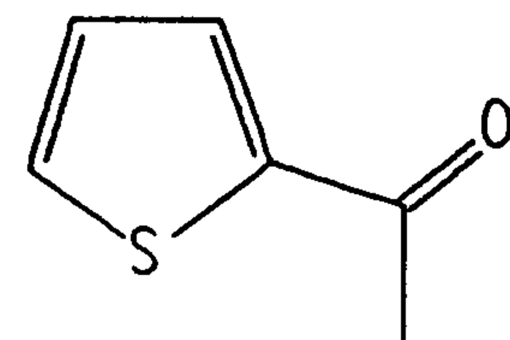


(79) LRI 1556

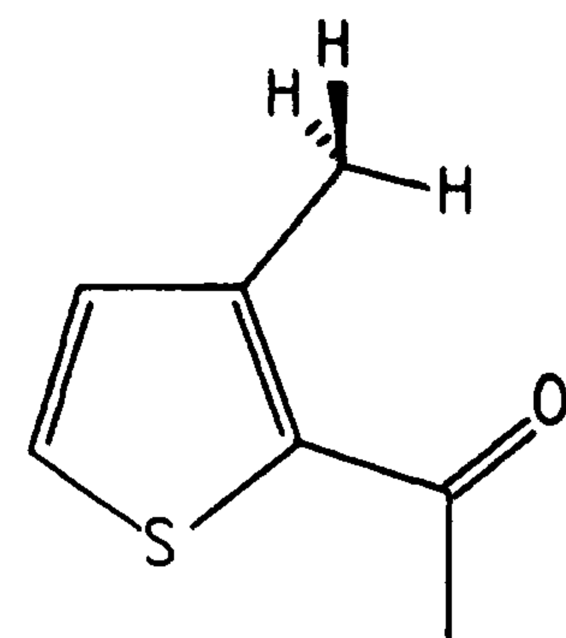


(74) LRI 1522

2-Acetylthiophenes

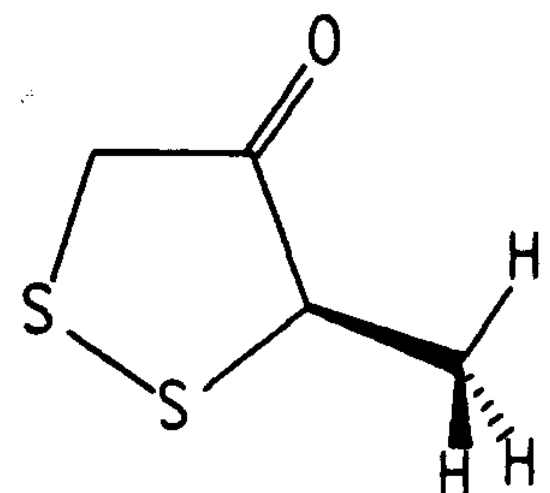


(121) LRI 1777

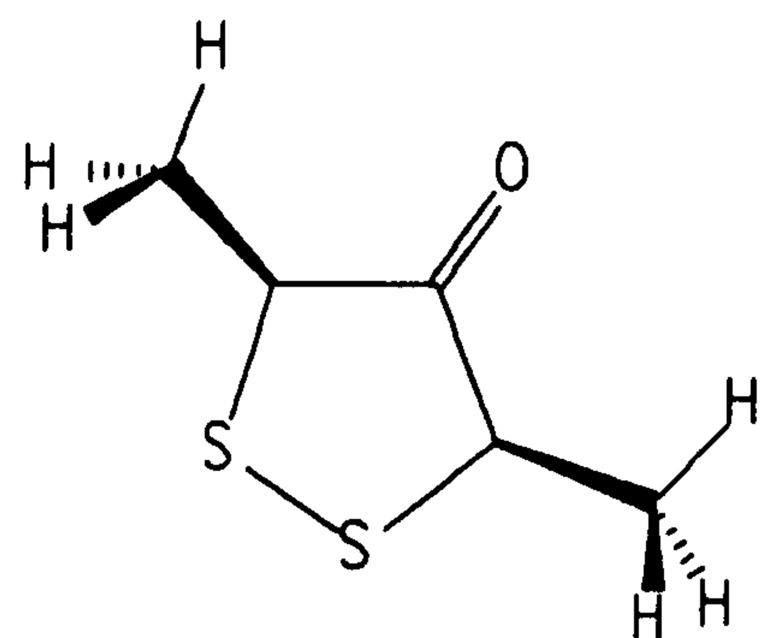


(119) LRI 1761

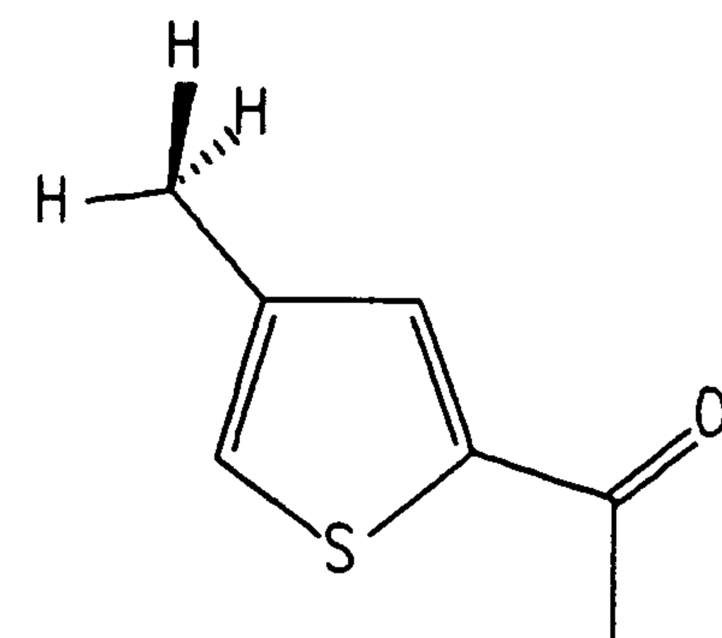
1,2-Dithiolan-4-ones



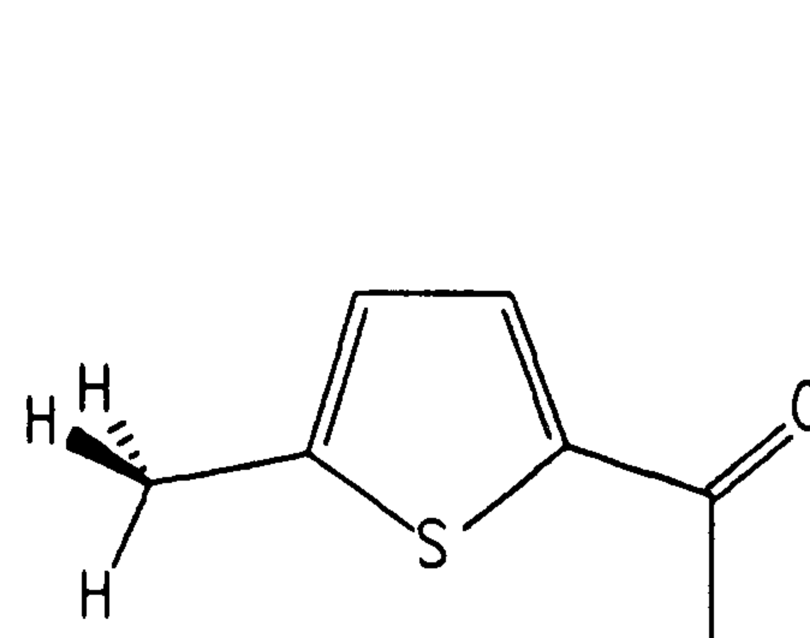
(101) LRI 1664



(83) LRI 1574

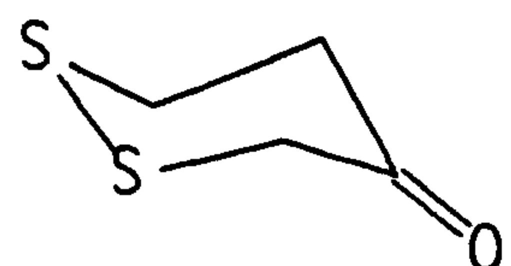


LRI 1890

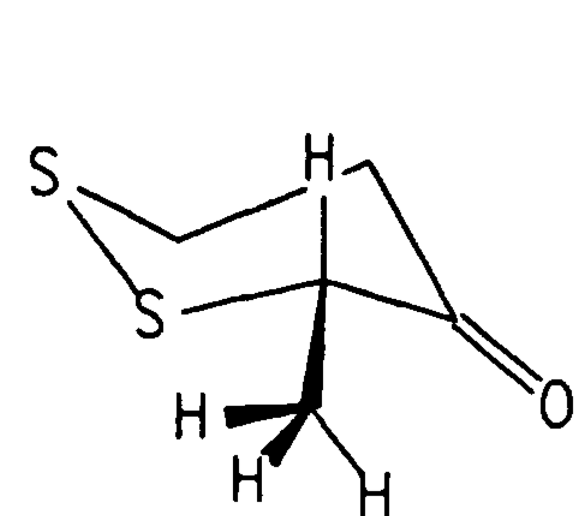


LRI 1874

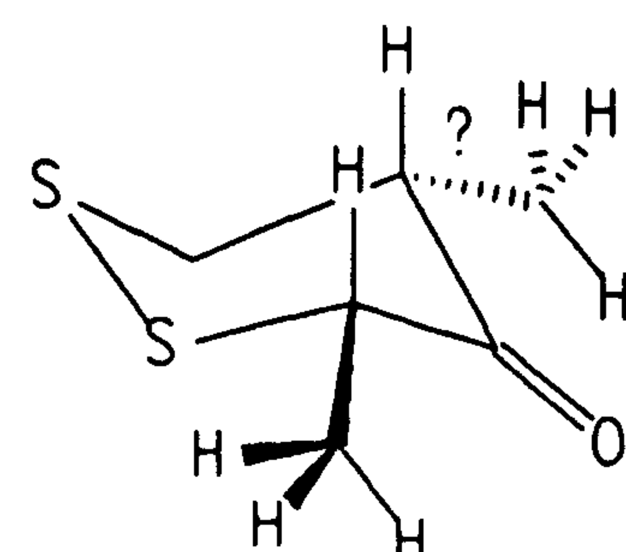
1,2-Dithian-4-ones



(143) LRI 1930



(139) LRI 1894



(132) LRI 1847

eluted before dihydro-3(2H)-thiophenone itself (**79**) and the dimethyl substituted compounds (**65**, **66**) eluted earlier still. This behaviour was consistent with the favoured identity for compound **75** of 4-methyl-3(2H)-thiophenone and may indicate that the two dimethyl compounds may be the 2,4-dimethyl-3(2H)-thiophenones.

For both the 1,2-dithiolan-4-ones and the dihydro-3(2H)-thiophenones the extension of a methyl substituent to give an ethyl group had the expected effect of increasing the elution time.

2.3.3 Aroma properties.

While it has been observed that a meaty odour is produced on heating cysteine with a reducing sugar (Morton *et al* 1960; Kiely *et al* 1960; Scanlan *et al* 1973), the effect of a lipid on this aroma has not been studied previously. The overall odour of both heated reaction mixtures was described as "sulphurous, rubbery" when assessed at close quarters. However, while the system containing only cysteine + ribose had some underlying meaty odour, the mixture containing phospholipid had a much more pronounced "cooked meat, beefy" aroma, which was most noticeable from a distance of several metres and as a lingering odour in the laboratory. The increase in the underlying "meatiness" when phospholipid was added to the reaction mixture corroborates the results of earlier experiments showing that the removal of phospholipid from meat reduced the meaty aroma (Mottram and Edwards 1983).

The effect of phospholipid on the formation of meat-like odours was further demonstrated by the GC effluent odour assessments; various types of meaty aroma were detected at 5.1, 10.7, 13.0, 20.5, 27.0, and 31.7 min. Only one of these (at 13.0 min, LRI~1300, most probably due to 2-methyl-3-furanthiol) was detected in the absence of phospholipid. Although a number of other compounds, found in the reaction mixture, are reputed to have meaty notes, e.g. thiazole (**22**), 2-methyl-3-thiophenethiol (**86**), 2-formylthiophene (**109**), and 3-methyl-1,2-dithian-4-one (**139**) (Fors 1983; Hartman *et al* 1984b; MacLeod 1986), their elution times do not correspond to those of the observed meaty odours.

This could be because the odour thresholds of these compounds are too high to allow their detection at the concentrations present in these reaction mixtures. However, the perceived aroma quality of chemicals is complex and is influenced by concentration, medium and other components. Many compounds change in character as the concentration increases above the odour threshold. The assessment of odour character is subjective and consequently universal agreement on odour quality is rarely obtained; meaty aromas are particularly difficult in this respect (MacLeod 1986). Thus, the interaction of phospholipid in the Maillard reaction between cysteine and ribose not only increased the perceived meatiness of the overall odour of the reaction mixture, but also increased the number of compounds possessing meaty aromas.

Due to the complexity of the chromatograms, it was not possible to assign odours to compounds unambiguously without peak trapping and further analysis; however, in some cases the aroma descriptions in the chromatograms were consistent with the known odours of the eluting compounds. The number of effluent odours with the descriptors, "sulphur", "garlic", "stench" reflected the sulphurous odour of the reaction mixtures. The compounds, 2-methyl-3-furanthiol (**29**) and 2-furanmethanethiol (**53**) are known to possess odours of beef broth and coffee respectively (Fors 1983), but each is strongly sulphurous at high concentrations. In both chromatograms, the 2-methyl-3-furanthiol was almost certainly responsible for the "acrid, sulphurous" odour at 13 min (LRI~1300) as well as the "meaty" odour arising as the concentration decreased on the tail of the peak, while 2-furanmethanethiol probably accounted for the "pungent, garlic" odours at 18 min (LRI~1420), although no coffee-like aroma was detected. The odour of 2-thiophenethiol (**80**) has been described as "very unpleasant, burnt caramellic and sulphurous" (Furia and Bellanca 1975) and its retention index coincides with the "pungent, garlic, sulphur" odours at 22.9 min (LRI~1560). The "catty, urine, garlic" odour at approx. 15.5 min (LRI~1350) eluted too early to be caused by 4-mercapto-4-methyl-2-pentanone, which is known to possess an extremely potent catty odour (Patterson 1969), but instead appears to correspond to 3-mercapto-2-pentanone (**43**).

The inclusion of phospholipid greatly increased the frequency and range of the odours eluting from the column. Additional odours described as "fatty", "green", and "aldehydic" reflected the

production of aliphatic compounds arising from the breakdown of the lipid. Some aromas could be attributed to compounds with known odour characteristics; the "floral, fruity, herbal, lemon" aroma at 16.5 min (LRI~1390) agreed with descriptions reported for nonanal ("green, oily, orange, rose") (Furia and Bellanca 1975; McGugan 1980), while the "fatty, green" odour detected at 10.2 min (LRI~1210) was probably due to 2-pentylfuran, which has been described as "pungent, green, fruity" (Fors 1983).

2.4 GENERAL DISCUSSION

The results described in Sections 2.2 and 2.3 list 109 volatile products of the Maillard reaction between glycine, ribose and phospholipid and 156 products from similar reactions using cysteine as the amino acid. Of these compounds, 45 were common to both systems. The nature of the compounds which dominated the headspace of these Maillard reactions was completely altered by the change of amino acid. The most abundant volatile products of the Maillard reaction between glycine and ribose were the alkylfurfurals, with other acyl furans and pyrazines also making major contributions to the chromatogram. While some of these compounds were detected among the products of the reaction between cysteine and ribose, they formed a relatively small proportion of the headspace volatiles. Instead, the headspace of the cysteine-containing systems was dominated by heterocyclic thiols, dihydro-3(2H)-thiophenones, acylthiophenes and bicyclic sulphur compounds.

Twelve acylfurans were identified among the headspace volatiles derived from the glycine-containing Maillard reaction; of these only three were included among the products of the reaction between cysteine and ribose. In particular, the alkyl furfurals, which were the dominant class of compounds in the glycine + ribose system, were present in very small amounts or were not detected; only 2-furfural was present in sufficient quantities to justify inclusion in Table 2.3a. However, the cysteine-containing Maillard reaction did produce 14 acylthiophenes, of which seven were analogous to acylfurans from the glycine-containing reaction mixture. Thus, it is likely that these thiophenes arose from similar pathways to the acylfurans, but that sulphur was incorporated at some stage in the reaction pathway.

Ten of the twelve acylfurans found among the headspace volatiles from glycine and ribose were also produced by heating ribose alone; those acylfurans with six or more carbon atoms were probably formed from the condensation of 2 and 3 carbon fragments, arising from the breakdown of the pentose sugar. The Strecker degradation of cysteine yields H_2S, which would react readily with these dicarbonyl and hydroxycarbonyl compounds to yield S-containing compounds, such as mercaptocarbonyls (Shu *et al* 1985d).

Mercaptoacetaldehyde is also produced directly by the Strecker degradation of cysteine. Condensation reactions between such S-containing fragments probably explain the formation of many of the acylthiophenes, dihydro-3(2H)-thiophenones, 1,2-dithian-4-ones and 1,2-dithiolan-4-ones, which contained six or more carbon atoms. Thus, it appears that H_2S and mercaptoacetaldehyde participate in reactions between sugar breakdown products to give a range of S-containing heterocyclic compounds.

The pyrazines, which were major components of the headspace from the reaction between glycine and ribose, were only present as minor products of the cysteine-containing reaction. The primary pathway for the formation of pyrazines is believed to involve the condensation of two aminocarbonyl compounds. Such compounds are formed from amino acids by Strecker degradation, which involves decarboxylation and dehydration reactions to yield, in the case of glycine, an aminocarbonyl compound and formaldehyde (Sec. 1.1.1.2). While the Strecker degradation of cysteine can give the analogous products, an alternative pathway results in the generation of H_2S, NH_3 and acetaldehyde. Thus, it is possible that, in the cysteine-containing systems, aminocarbonyls may be relatively minor products and the contribution made by pyrazines to the headspace of the reaction mixture reduced accordingly.

Despite the differences between the major headspace volatiles derived from these two Maillard systems, the effect of the inclusion of phospholipid was similar in both cases. Both the number and overall quantity of volatile compounds were increased with a concomitant increase in the number of individual odours detected by GC-aroma assessment. In addition to odours typical of lipid degradation products ("green", "fatty" etc), the inclusion of phospholipid added two "chicken-like" aromas to the glycine + ribose reaction and increased the number of odours described as "meaty" from the cysteine-containing system. Thus, these results concur with the hypothesis that the participation of phospholipid in the Maillard reaction plays an important part in the generation of the aroma and flavour of meat.

CHAPTER 3

QUANTITATIVE CHANGES IN VOLATILE COMPOUNDS DUE TO THE INTERACTION OF FOUR LIPIDS WITH THE MAILLARD REACTION BETWEEN CYSTEINE AND RIBOSE

3. QUANTITATIVE CHANGES IN VOLATILE COMPOUNDS DUE TO THE INTERACTION OF FOUR LIPIDS WITH THE MAILLARD REACTION BETWEEN CYSTEINE AND RIBOSE.

As described in the Introduction, work by Mottram and Edwards (1983) indicated that the phospholipid in meat was capable of interacting in the Maillard reaction to reduce the levels of certain heterocyclic products and to induce the meat-like aroma of cooked meat.

Subsequently, it was found that these results could be reproduced in model systems containing an amino acid, a reducing sugar and a phospholipid; the presence of phospholipid tended to reduce the levels of heterocyclic products (Whitfield *et al* 1988) and also altered the nature of the odour of the heated Maillard systems (Section 2; Salter *et al* 1988; Farmer *et al* 1989); where the amino acid used was cysteine, the presence of phospholipid added a persistent "meaty" note to the aroma.

The question remaining unanswered, both from the experiments on meat itself and those on model systems, was whether phospholipid itself was required to interact in the Maillard reaction or whether a small quantity of any lipid, eg. triglyceride, would have the same effect. An experiment was designed to address this question by comparing the effect of several lipids on the Maillard reaction between cysteine and ribose using aqueous model systems. Samples of triglyceride and phospholipid were extracted from bovine adipose tissue and muscle respectively. The lipids most likely to interact in the Maillard reaction are those present in the muscle itself where free amino acids and sugars are situated in aqueous solution. The extraction of beef triglyceride from the adipose tissue rather than muscle was felt to be justified as the fatty acid composition of adipose triglyceride is indistinguishable from that of intramuscular triglyceride (Christie 1978). Two commercially available phospholipids were also included; egg phosphatidylcholine (lecithin, PC) and egg phosphatidylethanolamine (cephalin, PE) provided a comparison between phospholipids with the two most common polar moieties. The PC also provided continuity with previous work (Whitfield *et al*

1988) while the PE had a fatty acid composition roughly comparable with that reported for beef phospholipid (Christie 1978).

The interaction between lipid and the Maillard reaction not only affects the formation of the largely heterocyclic products of this reaction but also influences the thermal degradation of the lipid. The reaction of lipid with protein, amino acids or ammonia is known to yield brown polymeric compounds and has been the subject of a number of reports (see Section 1.3). However, little information is available on the effect of Maillard reactants on the volatile products of lipid degradation. It is known that the presence of Maillard reactants during heating inhibits the development of rancidity in various food products during subsequent storage (Eichner 1981; Bailey *et al* 1987; Bailey 1988; Sec. 1.3.2); this antioxidative effect can also be demonstrated in model systems (Lingnert and Eriksson 1981). The suppression of certain volatile aldehydes has been used as a measure of the antioxidant activity of amino acids and sugars (Lingnert and Eriksson 1981); the reaction of aldehydes with amino groups to give imines and, eventually, polymeric substances may account for their depletion in food systems (Montgomery and Day 1965). However, the effect of Maillard reactants on the other volatile products of lipid thermal degradation has received little attention. The current experiment provided an opportunity to investigate the effect of cysteine and ribose on a range of ketones, alcohols and alkylfurans as well as saturated and unsaturated aldehydes.

The results of this experiment have been evaluated in two parts. Firstly, a study has been made of the effects of varying the lipid component on the major volatile products and overall aroma of the Maillard reaction between cysteine and ribose (Section 3.2). Secondly, the effects of cysteine and ribose on the thermal degradation products of the four lipids have been evaluated (Section 3.3).

Some of the results presented in this Chapter have been reported in two publications (Mottram and Salter 1989; Farmer and Mottram 1990a).

3.1 OUTLINE OF METHOD

Full details of the experimental method may be found in Section 6.

The four lipids investigated were beef triglyceride (BTG) and beef phospholipid (BPL), extracted from beef adipose tissue and *M. semimembranosus* respectively, egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE) from commercial sources. The nitrogen content was determined by micro-Kjeldahl analysis to ascertain purity. After saponification and methylation the fatty acid methyl esters were analysed by GC and GC-MS (electron impact and chemical ionization); the number of carbon atoms and double bonds in each fatty acid was calculated from the molecular weight (obtained by chemical ionization MS), while the position of double bonds was determined by coinjection with the authentic material or by comparison with the literature mass spectrum.

Cysteine and ribose were heated both with and without each of the four lipids in phosphate buffer at pH 5.6; each of the lipids was also heated alone under the same conditions as indicated in Table 3.1a. Reaction mixtures were prepared in quadruplicate.

Table 3.1a: Experimental plan

	With cysteine + ribose	Without cysteine + ribose (lipid alone)
Without lipid	Reaction mixture 1	2 (buffer) only
With beef triglyceride	3	4
With beef phospholipid	5	6
With egg PC	7	8
With egg PE	9	10

Lipid (30 mg) was placed in previously flamed 2.5 ml freeze-drying ampoules (numbered 3-10). Cysteine (5 mg ml^{-1}) and ribose (4.5 mg ml^{-1}) were dissolved in phosphate buffer (0.5M, pH 5.6) and 2 ml of this solution was added to all the odd-numbered ampoules. Nitrogen was blown over each of the samples for 2 min and the ampoules flame-sealed before being heated for 1 h at 140°C in an autoclave.

An internal standard containing alkanes (C_{10}-C_{24}, 10 ng each) and 1,2-dichlorobenzene (65 ng) was placed on freshly conditioned Tenax traps before collection. As each sample was opened prior to headspace collection, the odour was assessed by three flavour chemists in the laboratory and their comments noted. Each reaction mixture (2 ml) was diluted to 20 ml in phosphate buffer and stirred at 60°C while the headspace volatiles were swept onto the Tenax trap with a stream of N_2 (50 ml min^{-1}).

Analyses of the collected headspace volatiles were performed on a Carlo Erba 4130 GC coupled to a Finnigan 4000 mass spectrometer. The volatile components were desorbed on to the front of a CPWAX52CB fused silica capillary column (50 m x 0.32 mm ID), cooled by CO_2(s), prior to temperature programmed gas chromatography. The ion areas for selected volatile reaction products were determined by quantifying a characteristic ion from the mass spectrum of each compound; quantitation ions are listed in Appendix I. The ion areas were assessed in two ways:

(i) The unavailability of authentic samples for many of the heterocyclic compounds quantified made it impossible to measure the absolute quantities of each component; therefore, the results were calculated in terms of peak areas. In the complex chromatograms all the peaks did not separate completely and therefore quantitation was performed on characteristic ions. In order to obtain an indication of the relative contribution that each component made to the chromatogram, each ion area was calculated as the dividend of the area of the quantitated ion and its relative abundance in the mass spectrum. To take account of variations between replicates these areas were expressed relative to the area given by 1ng 1,2-dichlorobenzene to give relative peak areas (RPA). The RPA represents the contribution made by that compound to the ion chromatogram.

(ii) Authentic samples were available for most of the aliphatic compounds examined in Section 3.3. Therefore, it was possible to present the actual mass (ng) of each compound collected, in addition to the RPA data. A known amount of the compound was applied to a Tenax trap, together with internal standard, and a relative response factor for each compound obtained, enabling the calculation of the absolute quantities collected from the heated reaction mixtures.

3.2 A COMPARISON OF THE EFFECT OF A TRIGLYCERIDE AND THREE PHOSPHOLIPIDS ON THE VOLATILE PRODUCTS OF THE MAILLARD REACTION BETWEEN CYSTEINE AND RIBOSE: RESULTS AND DISCUSSION.

3.2.1 ANALYSIS OF LIPIDS

No phospholipid contamination (<<1%) was detected by thin layer chromatography in beef triglyceride purified from adipose tissue. Some other components were visible in the positions of mono- or diglycerides or free fatty acids, but concentrations were estimated to be <1% triglyceride.

The discarded triglyceride-containing fraction from lean muscle contained 1-3% phospholipid. As a large proportion of the total lipid eluted in this fraction, it is estimated that 3-10% of the phospholipids were lost with the triglyceride. Thin layer chromatography also showed the presence of low levels (1-3%) of triglyceride in the phospholipid fraction. As the purpose of the purification procedure was to obtain a sample of beef phospholipid in sufficient quantities to characterize and then use for inclusion in the Maillard reactions, and not to obtain an accurate lipid compositional analysis for that muscle, this degree of separation was considered adequate.

The micro-Kjeldahl analyses for nitrogen showed that the beef phospholipid sample contained 1.82% (SD = 0.089%) nitrogen, compared with a calculated value of 1.8 - 1.9%, suggesting that efforts to ensure the removal of proteinaceous contamination were successful. Nitrogen was not detected (< 0.05%) in the beef triglyceride sample.

The fatty acid compositions of the four lipids are shown in Table 3.2a. The most pronounced differences between the lipids lay in the proportion of polyunsaturated fatty acids, particularly those with three or more double bonds. Beef triglyceride contained a very low proportion of polyunsaturated fatty acids compared with the phospholipids, while among the phospholipids, the PE and beef phospholipid contained more polyunsaturated fatty acids with three or more double bonds than PC.

Table 3.2a. Fatty acid compositions for beef triglyceride (BTG), beef phospholipid (BPL), egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE).

Fatty acid	Method of identification[a]	Composition (% total fatty acids + aldehydes) BTG	BPL	PC	PE
14:0	I	3.2	-[b]	0.1	-
14:1		0.7	-	-	-
15:0	I	0.5	-	-	-
16:0	I	27.9	13.6	32.6	17.3
16:1 (Δ9)	I	3.1	1.3	1.0	0.5
17:0	I	1.5	0.6	0.2	0.2
17:1		0.9	0.6	-	-
18:0	I	18.3	11.9	12.8	25.6
18:1 (Δ9)	I	35.6	25.7	29.4	19.6
18:1 (Δ11)	I	2.7	1.6	1.0	0.9
18:2 (ω6)	I	0.6	7.1	16.1	13.4
18:3 (ω3)	I	0.4	2.8	-	-
20:3 (ω9)	L	-	1.0	-	-
20:3 (ω6)	L	-	1.9	0.4	0.4
20:4 (ω6)	I	-	7.3	3.7	13.3
20:4		-	0.9	-	-
20:5 (ω3)	t	-	4.8	-	-
22:4 (ω6)	t	-	0.3	0.2	0.8
22:5 (ω3)	I	-	4.6	1.1	3.5
22:6 (ω3)	I	-	1.3	0.7	2.2
Minor fatty acids		4.4	1.3	0.9	1.1
Aldehydes					
Hexadecanal	L	-	4.8	-	0.5
Heptadecanal	L	-	0.9	-	-
Octadecanal	L	-	4.8	0.1	0.6
Octadecenal	t	-	1.3	-	-
	Total	99.8	100.4	100.3	99.9
Σ saturated		51.4	26.1	45.6	43.1
Σ monounsat.		43.0	29.2	31.4	21.0
Σ diunsat.		0.6	7.1	16.1	13.4
Σ ≥3 double bonds		0.4	24.9	6.1	20.2
Σ aldehydes		-	11.8	0.1	1.1

a The identity of each fatty acid was established by comparison of the predicted molecular weight of its methyl ester with that obtained by GC-MS. The position of the double bonds was determined either by coinjection with the authentic material (I) or by comparison of the mass spectrum with those reported in the literature (L). Tentative suggestions only could be made concerning some of the isomers (t).

b - indicates that the fatty acid was not detected at greater than 0.1% of total fatty acids.

Eluting among the fatty acid methyl esters derived from the saponification of beef phospholipid were a series of long-chain aldehydes. These were presumed to arise from the plasmalogen forms of beef PC and PE, which are known to contain a considerable proportion of these phospholipids (Christie 1978). Low levels of these aldehydes were also detected from PC and PE. Plasmalogens are more readily cleaved by acid hydrolysis than by alkali saponification (Christie 1973) and it is likely that the amounts presented in Table 3.2a underestimate the true quantities present.

3.2.2 AROMA CHARACTERISTICS OF HEADSPACE VOLATILES.

A summary of the odour descriptions attributed to the five reaction mixtures containing cysteine and ribose is given in Table 3.2b. The major difference between the odours of these reaction mixtures was in the relative intensities of the various sulphurous and meaty notes. The reaction mixture containing triglyceride was not dissimilar to that from which all lipid was excluded, while

Table 3.2b: Summary of odour descriptions attributed to the headspace volatiles of the heated reaction mixtures containing cysteine and ribose.

Reaction mixture	Odour descriptions
1 Cysteine + ribose alone	Strong sulphurous, rubber, H_2S with a slight meaty (ham) note under.
3 Cysteine + ribose + beef triglyceride	Strong sulphurous, H_2S with some meaty (ham, roast, boiled) notes.
5 Cysteine + ribose + beef phospholipid	Distinctly meaty (chicken, roasted) under sulphurous and H_2S odours.
7 Cysteine + ribose + egg PC	Predominantly sulphurous, H_2S, rubber, but with meaty (ham, boiled) undertones.
9 Cysteine + ribose + egg PE	Very distinct meaty (strong, roast, lamb, boiled) aroma with some sulphurous, rubber odours. The most meaty.

all the phospholipid-containing systems had more intense meaty notes. Of particular note was a pleasant roast meat-like aroma which was discerned at a distance of 2-3 metres from the sample and which rapidly permeated the whole laboratory. This aroma was very pronounced in the reaction mixture containing PE, and to a lesser extent, beef phospholipid.

3.2.3 EFFECT OF LIPIDS ON SELECTED VOLATILE COMPOUNDS.

The major classes of compounds derived from the reaction between cysteine and ribose comprised furan and thiophenethiols, alkylthiophenes, acylthiophenes, thiophenones, thianones and thiolanones, bicyclic thiophenes, thiazoles, oxazoles and aliphatic thiols. More than 130 of these compounds were quantified and the most abundant members of each of the above classes, together with some additional compounds, such as 2-pentylpyridine and two furans, were selected for presentation in Table 3.2c. A total of 60 compounds are listed, along with their molecular weight and relative peak areas in the absence or presence of the four lipids.

The four lipids caused a variety of effects on the quantities of Maillard products and in order to rationalize the data, the compounds were classified according to the way the different lipids influenced their production. Thus each compound was assigned to one of the following categories and this information included in Table 3.2c:

- A Compounds formed only in the presence of lipid.
- B Compounds produced from the reaction of cysteine + ribose which were reduced by the presence of lipid, subdivided according to which lipids caused the greatest response.
  - a Phospholipids showed greater suppression than triglyceride.
  - b PE and beef phospholipid showed greater suppression than beef triglyceride and PC.
  - c Triglyceride showed greater suppression than phospholipids.

Table 3.2c: Relative peak areas for selected heterocyclic components formed from the Maillard reaction between cysteine and ribose in the absence and presence of four lipids.[a]

No	Compound	MW	Cysteine + ribose alone	+ BTG	+ BPL	+ PC	+ PE	Category[b]
Sulphur-containing compounds								
Alkylthiophenes and related compounds								
1	2-Methylthiophene	98	651 (341)[c]	420 (414)	1280 (1060)	217 (69)	546 (204)	D
2	4,5-Dihydro-2-methylthiophene	100	1220 (561)	889 (421)	1320 (646)	494 (78)	714 (161)	D
3	2-Ethylthiophene	112	367 (132)	236 (150)	949 (449)	267 (134)	323 (26)	D
4	2,5-Dimethylthiophene	112	2330 (875)	1770 (944)	4130 (1030)	2100 (731)	2240 (271)	D
5	2,3-Dimethylthiophene	112	597 (166)	337 (173)	966 (285)	586 (129)	530 (53)	D
6	2-Butylthiophene	140	0	0	33 (66)	176 (150)	176 (204)	A
7	2-Pentylthiophene	154	0	0	109 (112)	2700 (1970)	1230 (916)	A
8	2-Hexylthiophene	168	0	0	184 (290)	1220 (452)	436 (315)	A
9	2-Pentylthiapyran[e]	168	0	35 (33)	3150 (1880)	34700 (14800)	12500 (8250)	A
10	2-(1-Hexenyl)thiophene[e] (*cis/trans*)	166	0	0	0	65 (35)	42 (47)	A
11	2-(1-Hexenyl)thiophene[e] (*cis/trans*)	166	0	0	0	374 (217)	287 (308)	A
Acylthiophenes								
12	2-Formylthiophene	112	3450 (1280)	2460 (647)	1390 (233)	2340 (564)	1940 (411)	Bd
13	3-Acetylthiophene	126	1270 (282)	1070 (339)	901 (71)	1330 (222)	1170 (319)	Bd
14	2-Acetylthiophene	126	387 (121)	224 (60)	313 (71)	318 (53)	330 (119)	Bc
15	2-Propionylthiophene	140	3600 (592)	1950 (579)	2950 (234)	3170 (252)	3000 (768)	Bc
16	2-Formyl-3-methylthiophene	126	6940 (1790)	5830 (994)	2500 (1450)	5810 (1920)	4970 (1150)	Bd
17	3-Ethyl-2-formylthiophene[e]	140	3120 (826)	2030 (400)	1070 (583)	1980 (911)	1950 (352)	Bd
18	A dimethylformylthiophene[e]	140	880 (348)	403 (85)	237 (150)	425 (144)	691 (234)	Bd
19	A thienylethanal[e]	126	1150 (651)	1990 (829)	1570 (1010)	3420 (908)	1650 (791)	D
Heterocyclic thiols								
20	2-Furanmethanethiol	114	50200 (7090)	33400 (3560)	31600 (7530)	31000 (2490)	35900 (7130)	Bd
21	2-Methyl-3-furanthiol	114	30600 (3650)	12300 (3800)	4740 (1400)	8240 (1110)	7230 (2590)	Bb
22	2-Thiophenethiol	116	38300 (4390)	12300 (3730)	1230 (861)	17600 (3890)	5240 (3100)	Bb
23	3-Thiophenethiol[e]	116	2560 (1260)	1410 (458)	57 (66)	1220 (306)	536 (109)	Bb
24	2-Methyl-3-thiophenethiol[d]	130	32600 (4410)	2490 (2380)	83 (147)	6290 (1470)	920 (1390)	Bb
Thiophenones								
25	Dihydro-3(2H)-thiophenone	102	2280 (699)	1660 (502)	1520 (553)	1220 (292)	1300 (533)	Bd
26	Dihydro-2-methyl-3(2H)-thiophenone	116	38700 (12600)	27400 (7450)	36500 (15000)	24400 (2480)	26500 (5930)	Bd
27	*trans*-Dihydro-2,(4/5)-dimethyl-3(2H)-thiophenone[e]	130	4610 (1540)	2900 (287)	3050 (477)	3270 (511)	3390 (769)	Bd
28	*cis*-Dihydro-2,(4/5)-dimethyl-3(2H)-thiophenone[e]	130	5370 (1310)	4090 (593)	3830 (336)	4280 (427)	4340 (741)	Bd
29	Dihydro-(2/5)-ethyl-3(2H)-thiophenone[e]	130	5210 (1790)	2830 (1220)	3070 (1340)	3320 (507)	3180 (1130)	Bd

No	Compound	MW	Cysteine + ribose alone	+ BTG	+ BPL	+ PC	+ PE	Category
	Dithianones and trithianes							
30	1,2-Dithian-4-one[e]	134	904 (194)	937 (195)	1100 (500)	1030 (213)	693 (111)	C
31	3-Methyl-1,2-dithian-4-one[d]	148	559 (288)	406 (243)	690 (786)	674 (109)	507 (220)	C
32	trans-3,(5/6)-Dimethyl-1,2-dithian-4-one[e]	162	315 (90)	199 (137)	311 (111)	588 (90)	348 (125)	C
33	3-Methyl-1,2,4-trithiane	152	825 (504)	194 (135)	2080 (1300)	712 (198)	709 (396)	Bc
	Bicyclic compounds							
34	2,3-Dihydro-6-methyl-thieno[2,3c]furan	140	23600 (5200)	10400 (5100)	21000 (2300)	25000 (1810)	20600 (4420)	Bc
35	Thieno[2,3b]thiophene	140	7920 (2390)	1980 (761)	5410 (874)	6350 (934)	4680 (1720)	Bc
36	A methylthienothiophene[e]	154	2210 (720)	271 (126)	981 (335)	820 (115)	741 (340)	Bc
37	A dihydrothienothiophene[e]	142	57200 (29200)	25900 (8590)	56700 (5310)	71000 (6300)	52600 (12600)	Bc
38	A methyldihydro-thienothiophene[e]	156	15900 (2140)	2440 (1030)	7340 (4140)	5710 (1400)	5190 (2350)	Bc
	Thiazoles							
39	Thiazole	85	307 (198)	218 (141)	222 (125)	172 (25)	218 (140)	C
40	2-Methylthiazole[d]	99	247 (201)	242 (211)	168 (110)	136 (23)	146 (95)	C
41	4,5-Dimethylthiazole	113	502 (151)	406 (24)	285 (44)	438 (66)	389 (112)	C
42	Trimethylthiazole	127	1430 (419)	866 (253)	869 (328)	1450 (319)	1060 (469)	C
43	2-Acetylthiazole	127	980 (354)	772 (376)	879 (479)	1500 (528)	1010 (534)	C
	Mercaptocarbonyls							
44	2-mercapto-3-butanone[e]	104	19600 (6850)	19100 (8120)	8800 (2300)	10000 (1770)	9330 (3780)	Ba
45	2-mercapto-3-pentanone[e]	118	27100 (2710)	19600 (1850)	13300 (4330)	14400 (2800)	14300 (2580)	Ba
46	3-mercapto-2-pentanone[d]	118	28200 (1490)	21700 (1340)	13200 (3350)	14100 (2590)	13800 (2890)	Ba
47	1-Mercapto-3-pentanone[e]	118	1390 (646)	841 (243)	550 (473)	690 (92)	591 (223)	Ba
	Alkanethiols							
48	1-Heptanethiol	132	0	0	0	456 (351)	113 (126)	A
49	1-Octanethiol	146	0	0	0	341 (247)	104 (111)	A
	Compounds without sulphur							
50	2-furfural	96	1425 (481)	1140 (265)	1070 (394)	705 (121)	737 (159)	D
51	1-(2-furyl)-2-propanone[d]	124	2590 (694)	2510 (489)	2090 (122)	2710 (150)	2910 (593)	C
52	2-Pentylpyridine	149	0	26 (8)	279 (90)	5210 (2240)	429 (239)	A
	Pyrazines							
53	Methylpyrazine	94	478 (243)	555 (192)	548 (221)	300 (61)	570 (74)	C
54	Ethylpyrazine	108	1090 (261)	1140 (369)	1040 (377)	1280 (149)	1140 (237)	C
55	2,3-Dimethylpyrazine	108	362 (142)	393 (155)	369 (137)	427 (58)	392 (111)	C
56	2-Ethyl-5-methylpyrazine	122	217 (43)	187 (65)	179 (74)	226 (20)	205 (86)	C
57	Trimethylpyrazine	122	94 (52)	116 (55)	64 (37)	128 (15)	117 (59)	C

No	Compound	MW	Cysteine + ribose					Category
			alone	+ BTG	+ BPL	+ PC	+ PE	
	Oxazoles							
58	5-Ethyl-4-methyloxazole[d]	111	325 (131)	273 (39)	280 (48)	120 (17)	200 (57)	D
59	4-Ethyl-5-methyloxazole[d]	111	2180 (536)	1900 (96)	2060 (295)	997 (237)	1470 (411)	D
60	Trimethyloxazole	111	231 (121)	268 (104)	229 (92)	198 (47)	235 (56)	C

[a] The four lipids were: beef triglyceride (BTG), beef phospholipid (BPL), egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE).

[b] Compounds were assigned to categories (A, Ba-d, C, D) depending on the way in which the four lipids influenced their production, as defined in Section 3.2.

[c] Relative peak areas are expressed as the mean and (standard deviation), relative to the peak area given by 1 ng 1,2-dichlorobenzene = 100; values greater than 1000 are stated to three significant figures.

[d,e] Compounds for which the authentic compound was unavailable and whose identity was deduced either by comparison with a literature mass spectrum[d] or by interpretation of mass spectral and infra red data[e] as described previously (Sec. 2.3; Farmer *et al* 1989). Compounds 23, 44 and 45 were not reported in Section 2.3; compound 23 was identified by comparison with the mass spectral data cited by Heller and Milne (1978) while compounds 44 and 45 were identified by interpretation of the following mass spectra:

No. 44: 43, 61(48), 104(20), 60(18), 35(13), 59(9), 45(6), 58(3), 62(2)

45: 57, 61(51), 118(17), 43(10), 41(8), 60(8), 35(7), 59(5), 56(5), 58(5)

- d Triglyceride caused similar reductions to phospholipids.
- C Compounds largely unaffected by lipid.
- D Compounds not included in previous categories.

The aim of the experiment was to compare the effect of the four lipids on the quantities produced of the different classes of compounds formed by the Maillard reaction between cysteine and ribose and on the compounds within these classes. Therefore, the comparisons of most interest were those between the reaction mixtures containing no lipid, triglyceride or phospholipid and secondly, any differences in effect observed between the three phospholipids studied.

It was apparent that members of a given class of compounds generally showed the same pattern of response to the presence of the different lipids. The first part of the discussion therefore examines each of the typical patterns of response which the four lipids engendered in the production of volatile components and those compounds falling within each category. Mechanisms by which lipids may elicit these effects are then discussed.

For ease of comparison normalized values are presented in the text. For most categories the values for each compound have been divided by the amount given by cysteine + ribose alone; however, for Category A the amount derived from the PC-containing system was taken as the reference.

3.2.3.1 Compounds formed only in the presence of lipid (A).

The greatest differences between the effects of the four lipids were observed among those compounds which required lipid precursors for their formation. These compounds included 2-pentylpyridine, 2-alkylthiophenes, alkenylthiophenes, a pentylthiopyran and alkanethiols. All these compounds followed a similar pattern; much greater quantities were produced by the phospholipid-containing systems than by those containing triglyceride, which gave little or none of these compounds. Amongst the three phospholipids, by far the greatest amount of

Compounds in Category A

No.	Compound	alone	Cysteine + ribose +BTG	+BPL	+PC	+PE
52	2-Pentylpyridine	0	0.005	0.054	1	0.082
6	2-Butylthiophene	0	0	0.19	1	1
7	2-Pentylthiophene	0	0	0.040	1	0.46
8	2-Hexylthiophene	0	0	0.15	1	0.36
9	2-Pentylthiapyran	0	0.001	0.091	1	0.36
10	2-(1-Hexenyl)-thiophene	0	0	0	1	0.65
11	2-(1-Hexenyl)-thiophene	0	0	0	1	0.77
48	1-Heptanethiol	0	0	0	1	0.25
49	1-Octanethiol	0	0	0	1	0.30

these compounds was obtained from the Maillard system containing PC, with beef phospholipid and PE giving intermediate quantities.

As discussed in a previous paper (Whitfield *et al* 1988), 2-pentylpyridine can arise from the reaction of 2,4-decadienal with ammonia. The ammonia is thought to result from the Strecker degradation of cysteine, while 2,4-decadienal was produced in differing quantities by the thermal degradation of the four lipids when heated alone. Appreciably more 2,4-decadienal was produced by PC than by the other lipids; the relative amounts for beef triglyceride : beef phospholipid : PC : PE were 1.4 : 1.3 : 64 : 1 (Section 3.3). Thus, the origin of large quantities of 2-pentylpyridine in the volatile products of the PC-containing Maillard system is clear.

The 2-alkylthiophenes could result from the action of H_2S on the corresponding furans at high temperature, as suggested by Vernin and Parkanyi (1982). A more probable route is from the reaction of H_2S with dienals, giving 2-hexylthiophene from 2,4-decadienal and the 2-pentyl and 2-butyl homologues from 2,4-nonadienal and 2,4-octadienal, respectively. However, the relative amounts of the three alkylthiophenes in the PC-containing mixtures do not reflect the extreme preponderence of 2,4-decadienal over the other dienals found in the reaction mixtures. A compound with a mass spectrum very similar to that of 2-hexylthiophene, but with a retention

time close to that of 2-heptylthiophene, was tentatively identified as 2-pentylthiapyran (Sec. 2.3.1; Mottram and Salter 1989). 2-Pentylthiapyran was formed in much greater quantities than its butyl and propyl homologues which were also detected but in lesser amounts than the isomeric thiophenes. Thus, the reaction of H_2S with 2,4-decadienal may favour the formation of the thiapyran while 2,4-nonadienal and 2,4-octadienal tend to react to give the alkylthiophenes. Possible routes of formation for 2-pentylpyridine, 2-hexylthiophene and 2-pentylthiapyran from 2,4-decadienal were shown in Figure 2.3L.

The two isomers of 2-(1-hexenyl)thiophene were also identified; these are both found at higher levels in the presence of the two egg phospholipids than the beef phospholipid.

1-Heptanethiol and 1-octanethiol were only present in the Maillard systems to which phospholipids had been added. In common with the hexenylthiophenes, the amounts produced were considerably raised in the PC, and to a lesser extent, in the PE-containing Maillard systems. The thermal degradation of PC yielded larger quantities of 1-heptanol and 1-octanol than PE, while for the corresponding aldehydes the reverse was true. This would suggest that the alkanethiols were formed by the action of H_2S on the corresponding alcohols rather than from the aldehydes. Furthermore, while heptanol and octanol were the major lipid derived alcohols, the most abundant aldehydes were generally nonanal and hexanal.

3.2.3.2 Compounds produced from the reaction of cysteine + ribose which were reduced by the presence of lipid (B).

In agreement with the results of previous studies (Mottram and Edwards 1983; Whitfield *et al* 1988) many Maillard reaction products showed marked reductions on the addition of lipid. Again several distinct patterns of effect were observed within this category:

a) Phospholipids showed greater suppression than triglyceride.

The four mercaptoketones tended to be reduced only slightly by the addition of triglyceride and reduced considerably by all of the phospholipids. These compounds were probably formed by the

Compounds in Category Ba

No.	Compound	alone	Cysteine + ribose +BTG	+BPL	+PC	+PE
44	2-Mercapto-3-butanone	1	0.97	0.45	0.51	0.48
45	2-Mercapto-3-pentanone	1	0.72	0.49	0.53	0.53
46	3-Mercapto-2-pentanone	1	0.77	0.47	0.50	0.49
47	1-Mercapto-3-pentanone	1	0.61	0.40	0.50	0.43

reaction of H_2S with dicarbonyl or α,β-unsaturated carbonyl compounds (Boelens *et al* 1975; Takken *et al* 1976; Badings *et al* 1976). The removal of H_2S from the system by reaction with the polyunsaturated fatty acids of the phospholipids may be an explanation of the reduction of mercaptoketones in the presence of phospholipids.

b) PE and beef phospholipid showed greater suppression than beef triglyceride and PC.

Compounds in Category Bb

No.	Compound	alone	Cysteine + ribose +BTG	+BPL	+PC	+PE
21	2-Methyl-3-furanthiol	1	0.40	0.15	0.27	0.24
22	2-Thiophenethiol	1	0.32	0.032	0.46	0.14
23	3-Thiophenethiol	1	0.55	0.022	0.48	0.21
24	2-Methyl-3-thiophenethiol	1	0.076	0.0025	0.19	0.028

The furan and thiophenethiols are of special interest due to the key role they are thought to play in the flavour of meat (Gasser and Grosch 1988). 2-Methyl-3-furanthiol and the analogous thiophene have aromas described as "roasted meat" (Evers *et al* 1976; van den Ouweland and Peer 1975), while compounds with the sulphur in the 2-position give aromas which are "burnt", "sulphurous" and "rubbery" but not meat-like (Evers *et al* 1976).

All the thiol substituted compounds were markedly reduced in the presence of beef triglyceride and PC, and suppressed even more in the presence of beef phospholipid and PE. At first glance, these results seem at odds with the observation that cysteine + ribose mixtures containing these latter two lipids gave the most intense meaty aromas. However, in the model systems described, 2-methyl-3-furanthiol occurred at much higher concentrations than those found in meat (Gasser and Grosch 1988) and at high concentrations the

odour quality changes from "meat-like" to "pungent" and "sulphurous". Thus, it may be that in these model systems the phospholipids contribute to the generation of meaty aromas, in part, by suppressing the production of these very potent odour chemicals to the low levels necessary for them to possess a pleasant meaty character. However, it is difficult to reconcile the determined levels of this key odour compound with the similarity in odour between those reaction mixtures containing triglyceride and cysteine + ribose alone, or with the marked difference in aroma between the reaction mixtures containing PE and PC; clearly, other factors are involved and meaty character is not determined by these compounds alone.

The various sulphides and disulphides of these compounds described in Section 2.3.1 were not detected among the headspace volatiles of the reaction mixtures involving the different lipids. Subsequent investigation suggested that this may have been due to the higher buffering capacity of the phosphate buffer used in this experiment; instead of the 0.2M phosphate buffer used in the earlier experiment, a 0.5M buffer was utilized to minimise the pH drop during reaction. The pH dependency of these compounds has been the subject of more recent studies (Farmer and Mottram 1990b).

c) **Triglyceride showed greater suppression than phospholipids.**

Compounds in Category Bc

No.	Compound	Cysteine + ribose alone	+BTG	+BPL	+PC	+PE
34	2,3-Dihydro-6-methyl-thieno[2,3c]furan	1	0.44	0.89	1.06	0.87
35	Thieno[2,3b]thiophene	1	0.25	0.68	0.80	0.59
36	A Methylthieno-thiophene	1	0.12	0.44	0.37	0.34
37	A Dihydrothieno-thiophene	1	0.45	0.99	1.24	0.92
38	A Methyldihydro-thienothiophene	1	0.15	0.46	0.36	0.33
33	3-Methyl-1,2,4-trithiane	1	0.24	2.52	0.86	0.86
14	2-Acetylthiophene	1	0.58	0.81	0.82	0.85
15	2-Propionylthiophene	1	0.54	0.82	0.88	0.83

A series of bicyclic sulphur compounds were identified in the cysteine - ribose reaction (see Sec. 2.3.1). These included 2,3-dihydro-6-methylthieno[2,3c]furan (kahweofuran) and isomers of thienothiophene, as well as a series of methyl-substituted thienothiophenes and dihydrothienothiophenes. Each of these compounds reacted to the inclusion of the four lipids in the same way, as is illustrated by the selection of these compounds for which data are presented in Table 1. The amounts of some of these substances were reduced somewhat by phospholipids, but without exception they were all strongly suppressed by the presence of triglyceride. As the route of formation of these compounds has not been elucidated, it is not clear why triglyceride elicited this effect.

3-Methyl-1,2,4-trithiane was decreased by triglyceride but, interestingly, also showed an increase in the presence of beef phospholipid. This compound may arise by a similar route to the isomeric 3,5-dimethyl-1,2,4-trithiolane which is formed from ethanal and H_2S, both of which are breakdown products of cysteine (Boelens *et al* 1974).

As hydrogen sulphide is an important intermediate for all these compounds, it might have been expected that those lipids high in polyunsaturated fatty acids would have suppressed formation most by competing for reaction with H_2S. It is possible that the volatilization of these compounds was hindered by the physical characteristics of the triglyceride-containing reaction mixture. Although there was no greater suppression by triglyceride with decreasing volatility of these compounds, some of these substances were among the least volatile of the reaction products detected and therefore might have been most affected by the fact that unlike the phospholipids, triglyceride tended to form a separate layer during headspace collection.

The two acylthiophenes which fall into this category show only minor effects from the inclusion of any of the lipids; the remaining acylthiophenes fit better into Category Bd.

d) Triglyceride caused similar reductions to phospholipids.

Compounds in Category Bd

No.	Compound	Cysteine + ribose alone	+BTG	+BPL	+PC	+PE
12	2-Formylthiophene	1	0.71	0.40	0.68	0.56
13	3-Acetylthiophene	1	0.84	0.71	1.05	0.92
16	2-Formyl-3-methyl-thiophene	1	0.84	0.36	0.84	0.72
17	2-Ethyl-2-formyl-thiophene	1	0.65	0.34	0.63	0.63
18	A dimethylformylthiophene	1	0.46	0.27	0.48	0.79
25	Dihydro-3(2H)-thiophenone	1	0.73	0.67	0.54	0.57
26	Dihydro-2-methyl-3(2H)-thiophenone	1	0.71	0.94	0.63	0.68
27	*trans*-Dihydro-2,(4/5)-di-methyl-3(2H)-thiophenone	1	0.63	0.66	0.71	0.74
28	*cis*-Dihydro-2,(4/5)-di-methyl-3(2H)-thiophenone	1	0.76	0.71	0.80	0.81
29	Dihydro-(2/5)-ethyl-3(2H)-thiophenone	1	0.54	0.59	0.64	0.61
20	2-Furanmethanethiol	1	0.67	0.63	0.62	0.72

In contrast to the compounds in categories Ba, Bb and Bc, which showed marked distinctions between the effects of triglyceride and the phospholipids, there were a number of compound classes for which only small variations between the effects of different lipids were observed.

There was a small but consistent tendency for all the lipids to reduce the amounts of the acyl thiophenes, with beef phospholipid usually causing the most suppression. Dimethylformylthiophene exhibited this effect to the greatest degree.

Similarly, the effects of lipid on the amounts of 3(2H)-thiophenones were relatively small, but there was a consistent trend towards reduced levels in the presence of all the lipids.

2-Furanmethanethiol was equally reduced by all the four lipids; this compound is thought to contribute to the characteristic aroma of coffee (Stoll 1967; Tressl and Silwar 1981) but is also a constituent of the volatiles of cooked beef and chicken.

3.2.3.3 Compounds largely unaffected by lipid (C).

Compounds in Category C

No.	Compound	Cysteine + ribose alone	+BTG	+BPL	+PC	+PE
30	1,2-Dithian-4-one	1	1.04	1.22	1.14	0.77
31	3-Methyl-1,2-dithian-4-one	1	0.73	1.23	1.21	0.91
32	*trans*-3,(5/6)-dimethyl-1,2-dithian-4-one	1	0.63	0.99	1.87	1.10
39	Thiazole	1	0.71	0.72	0.56	0.71
40	2-Methylthiazole	1	0.98	0.68	0.55	0.59
41	4,5-Dimethylthiazole	1	0.81	0.57	0.87	0.77
42	Trimethylthiazole	1	0.61	0.61	1.01	0.74
43	2-Acetylthiazole	1	0.79	0.90	1.53	1.03
53	Methylpyrazine	1	1.16	1.15	0.63	1.19
54	Ethylpyrazine	1	1.05	0.95	1.17	1.05
55	2,3-Dimethylpyrazine	1	1.09	1.02	1.18	1.08
56	2-Ethyl-5-methylpyrazine	1	0.86	0.82	1.04	0.94
57	Trimethylpyrazine	1	1.23	0.68	1.36	1.24
60	Trimethyloxazole	1	1.16	0.99	0.86	1.02
52	1-(2-Furyl)-2-propanone	1	0.97	0.81	1.05	1.12

The formation of certain classes of compounds appeared largely unaffected by the inclusion of lipid in the Maillard reaction mixture. Interestingly, the three dithianones were included in this group. It has been proposed that the mechanisms of formation of these and the thiophenones may be related (Hartman *et al* 1984b). However, there were consistent differences between these two classes in the effect of lipid on the amounts formed.

Certain of the thiazoles showed some reduction on the addition of lipid, especially PC (in agreement with previous results, Whitfield *et al* 1988); however, the quantities were small and any differences between the four lipids were within the range of the standard deviation and showed no clear trends.

Mottram and Edwards (1983) reported that the removal of phospholipids from meat increased the pyrazines detected among the volatile products, while MacLeod and Ames (1987) found no overall increase in pyrazines in defatted meat, although certain

individual pyrazines were enhanced. In model systems based on cysteine + ribose, only very small effects on pyrazines have been observed, in this study and previously (Whitfield *et al* 1988). In both cases methyl pyrazine alone showed a reduction in the presence of phospholipid, and only PC gave this effect. The absence of any consistent reductions of pyrazines on the addition of lipid is probably because, in an acidic medium, pyrazines are thought to be formed from the Strecker degradation of amino acids with dicarbonyls via the condensation of the resulting aminoketones (Tsuchida *et al* 1976); free NH_3, which would be vulnerable to reaction with the products of lipid degradation, is not required. Pyrazines make an extremely small contribution to the volatile products of the Maillard reaction between cysteine and ribose; this may be due to the fact that, while for most amino acids Strecker degradation yields aminoketones and an aldehyde, for cysteine an alternative and favoured branch of this pathway generates H_2S, NH_3, ethanal and the original dicarbonyl. Thus, aminoketones are probably only minor products of the Strecker degradation of cysteine.

3.2.3.4 Compounds not included in previous categories (D).

Compounds in Category D

No.	Compound	Cysteine + ribose alone	+BTG	+BPL	+PC	+PE
50	2-Furfural	1	0.80	0.75	0.49	0.52
58	5-Ethyl-4-methyloxazole	1	0.84	0.86	0.37	0.62
59	4-Ethyl-5-methyloxazole	1	0.87	0.94	0.46	0.67
19	A Thienylethanal	1	1.73	1.37	2.97	1.43
1	2-Methylthiophene	1	0.65	1.97	0.33	0.84
2	4,5-Dihydro-2-methyl-thiophene	1	0.73	1.08	0.40	0.59
3	2-Ethylthiophene	1	0.64	2.59	0.73	0.88
4	2,5-Dimethylthiophene	1	0.76	1.77	0.90	0.96
5	2,3-Dimethylthiophene	1	0.56	1.62	0.98	0.89

Surprisingly, 2-furfural showed much more difference between the effects of the various lipids than did the product of its reaction with H_2S, 2-furanmethanethiol; the final concentration was

approximately halved by the addition of PC or PE, while beef triglyceride and beef phospholipid gave lesser degrees of suppression. As discussed previously (Whitfield *et al* 1988), the addition of lipid would not be expected to affect the formation of furfural from ribose; however, in the presence of lipid degradation products further reactions may occur to form polymeric material or volatile fragmentation products (Shibamoto 1977).

The two ethylmethyloxazoles showed a similar pattern of lipid effect to 2-furfural, albeit less pronounced, unlike trimethyloxazole (Category C) which was unaffected by the presence of any of the lipids. Trimethyloxazole may be derived from the reaction of cysteine with 2,3-butanedione (Hartman and Ho 1984) or possibly from the condensation of aminoketones and acetaldehyde arising from the Strecker degradation of cysteine (Vernin and Parkanyi 1982). However, as mentioned previously, aminoketones may only be minor products of the Strecker degradation of cysteine. Both the ethylmethyloxazoles could be formed by the Strecker degradation of cysteine with 2,3-pentanedione followed by reaction with formaldehyde, by the mechanism descibed by Vitzthum and Werkhoff (1974b) but a more likely mechanism is by the reaction of formaldehyde and ammonia with the hydroxyketone (Mottram 1990).

The formation of thienylethanal was unusual in that it was produced by the reaction mixture containing only cysteine and ribose, but its quantity was increased by the presence of all the lipids, especially PC; either there is more than one route of formation of this compound or a key precursor can be formed both from the Maillard system and lipid.

In contrast to the long-chained alkylthiophenes, the only consistent effect shown by the ethyl and dimethyl thiophenes were increased levels resulting from the addition of beef phospholipid.

3.2.4 MECHANISMS OF INTERACTION OF LIPID IN THE MAILLARD REACTION.

Although the interaction of lipids in the Maillard pathways responsible for the formation of volatile compounds has received little attention, studies on related topics suggest several routes by which phospholipids may interact in the Maillard reaction;

these have been discussed in full in Section 1.3. In a study of the browning reaction of various unsaturated fatty acids heated with either choline or ethanolamine, it was shown that resultant colour intensity increased with degree of fatty acid unsaturation in the presence of both these compounds but that, for the same fatty acid, choline gave more browning than ethanolamine (Husain *et al* 1986). However, ethanolamine showed much greater reactivity than choline when heated with the conjugated carbonyl derivative of methyl linoleate, due to reaction of this group with the free amino group to yield a Schiff's base and thence coloured products.

Lipid-derived unsaturated aldehydes and hydroxyketones also cause browning of proteins, again via the formation of Schiff bases, while free radicals derived from peroxidized lipids are known to react with the amino acids of proteins in dry mixtures, especially those with S or N-containing groups (Pokorny 1981; Sec. 1.3.1). However, this latter effect may be attenuated by the antioxidative effect of some Maillard intermediates and products which is thought to involve the inactivation of radical-producing hydroperoxides (Eichner 1981; Lingnert and Eriksson 1981; Bailey *et al* 1987; Sec. 1.3.2). The extent to which free radical reactions would be important in the aqueous systems of the current work is questionable; a water activity of 0.75 has been shown to reduce the occurrence of protein radicals formed by oxidizing lipid by a factor of four (Schaich and Karel 1975).

Dicarbonyl compounds and alkenals have been shown to react with H_2S to give a range of compounds, including mercaptocarbonyls and alkylthiophenes (Boelens *et al* 1975; Badings *et al* 1976). The oxidation of lipids can yield longer-chain homologues of these carbonyl compounds; as unsaturated fatty acids are the most readily oxidized, they are likely to be the most reactive with free H_2S.

Thus, the most likely routes by which lipid could interact in the Maillard reaction may be summarized as follows:

a the reaction of lipid-derived carbonyl products with the amino groups of cysteine and the ammonia produced by its Strecker degradation.

b the reaction of the NH_2 group of ethanolamine (in PE) with sugar-derived carbonyl compounds.

c the interaction of free radicals from peroxidized lipids in the Maillard reaction.

d the reaction of hydroxy and carbonyl lipid oxidation products with free H_2S.

The results descibed in this Chapter not only confirm that lipids interact in the Maillard reaction to modify the volatile aroma compounds produced, but also demonstrate marked dissimilarities between the behaviour of the four lipids. The most noticeable distinction was between the effect of triglyceride and the three phospholipids; with a few exceptions those compounds whose mechanisms of formation were susceptible to lipid intervention were more affected by the presence of a phospholipid than the beef triglyceride. This differentiation was most pronounced in the volatile compounds requiring the presence of lipid for their formation but was also very marked in some Maillard products, especially the mercaptocarbonyls, 3-furanthiols and thiophenethiols. Both these effects can be explained by the paucity of highly unsaturated fatty acids in the beef triglyceride relative to the phospholipids; the triglyceride fatty acids contained less than 2% with 2 or more double bonds, compared with at least 20% for the phospholipids. Thus, triglyceride lacks the most reactive precursors both for the formation of the long chained heterocyclic compounds and for reacting with and absorbing the active free molecules, such as NH_3 and H_2S, formed in the Maillard reaction. The participation of triglyceride in the Maillard reaction may also differ from that of phospholipid due to the fact that triglyceride is less miscible with the aqueous Maillard reactants than the phospholipids; it is possible that such physical effects limit the participation of triglycerides in the Maillard reaction in both model systems and meat. The same division was noted between the odours of the reaction mixtures; the odour of the triglyceride-containing reaction mixture was very similar to that of cysteine + ribose alone, while all those containing a phospholipid had some "meaty" character in addition to the sulphurous notes.

In addition to the clear difference between the triglyceride and the phospholipids, there were also variations in the way that the three phospholipids interacted in the Maillard reaction. A comparison of the two purified classes of phospholipid represented in this study, egg PC and PE, showed that, despite the greater impact of PC upon the long chained heterocyclics and thiols, PE conferred the most pleasant meaty note on these model systems. Egg PC clearly donated important precursors for the formation of 2-pentylpyridine, 1-alkanethiols, 2-alkylthiophenes etc., while PE and beef phospholipid were generally most effective at suppressing certain Maillard products, especially heterocyclic thiols and some of the acylthiophenes.

One characteristic shared by PE and beef phospholipid was the presence of the ethanolamine free amino group. The concentration of these was approx. 20mM in egg PE, and ca. 8mM in beef phospholipid, compared with 41mM of cysteine amino groups; thus these groups could have offered some competition for reactive carbonyl compounds. It seems more likely however that the similarities in effect between PE and beef phospholipid were related to the very high proportions (>20%) of highly unsaturated fatty acids containing 3 or more double bonds; less than 7% of the PC fatty acids fell into this category. Free H_2S and NH_3, both derived from the Strecker degradation of cysteine, are key reactants in the formation of many flavour compounds. It seems probable that polyunsaturated fatty acids and their thermal degradation products compete with other components for H_2S and NH_3 as well as other amino compounds. This may have resulted in the suppression of key meat aroma compounds, such as 2-methyl-3-furanthiol to concentrations where their odour is pleasant, while the concentrations of the mercaptocarbonyls, which frequently have highly objectionable odours, were also reduced, possibly to levels at which they contributed less to the overall character of the aroma.

The difference in effect between PC and PE could be due to the dissimilarities in either fatty acid composition or polar moiety. Detailed explanations of these effects await investigations of the individual interactions occurring between lipid degradation products and Maillard intermediates.

3.3 EFFECT OF CYSTEINE AND RIBOSE ON THE VOLATILE THERMAL DEGRADATION PRODUCTS FROM A TRIGLYCERIDE AND THREE PHOSPHOLIPIDS: RESULTS AND DISCUSSION.

The previous Section established that the four lipids studied (beef triglyceride, beef phospholipid, egg phosphatidylcholine and phosphatidylethanolamine) exhibited certain definite and distinct effects on the volatile products and aromas generated by the Maillard reaction between cysteine and ribose. However, in addition to this effect, it is known that Maillard products can exert an antioxidative effect on lipids (Sec. 1.3.2). Thus, it was also of interest to examine the effect of cysteine and ribose on the major volatile products derived from the thermal oxidation of the four lipids.

The experimental design was described in Section 3.1 and the fatty acid composition of the lipids was given in Section 3.2.1 (Table 3.2a).

Each of the four lipids heated alone yielded saturated and unsaturated alcohols, aldehydes, ketones and alkylfurans as major volatile products. Tables 3.3a and 3.3b show the effect on these compounds of including the Maillard reactants, cysteine and ribose during heating. The results of quantitation are presented both in terms of relative peak areas (RPA; Table 3.3a) and mass (ng) of compound collected (Table 3.3b).

3.3.1 AROMA CHARACTERISTICS OF HEATED LIPIDS

The odour descriptions of the reaction mixtures containing cysteine and ribose and each of the four lipids were presented in Table 3.2b in Section 3.2.2. However, the odours of these lipids heated alone were also of some interest; a summary of these odour descriptions is given in Table 3.3c.

These results show that purified beef triglyceride has only a faint "fatty" odour and does not possess any meaty or species-specific notes. Wasserman and Spinelli (1972) found that, although

Table 3.3a: Relative peak areas[a] for selected lipid degradation products formed from various lipids in the absence and presence of cysteine and ribose.

	Lipid[b]:	BTG		BPL		PC		PE	
	Cysteine + ribose:	-	+	-	+	-	+	-	+
No. Compound									
1. Hexanal	mn	8310	3900	22000	11000	20600	17100	52900	17100
	sd	(76)	(1870)	(9000)	(1680)	(2820)	(5840)	(8760)	(4350)
2. Heptanal	mn	6740	2640	11100	5040	9820	10400	32600	6290
	sd	(649)	(697)	(2910)	(646)	(190)	(3700)	(6670)	(4460)
3. Octanal	mn	6110	392	14100	860	12300	4790	29700	1290
	sd	(746)	(106)	(5850)	(343)	(511)	(2850)	(9310)	(1210)
4. Nonanal	mn	11600	3100	19000	3930	35000	17200	26600	2050
	sd	(1170)	(1110)	(6400)	(840)	(11900)	(2940)	(5350)	(434)
5. Decanal	mn	864	324	1640	887	5430	1260	2600	535
	sd	(327)	(90)	(651)	(628)	(1950)	(386)	(1040)	(298)
6. Hexadecanal	mn	0	0	14000	6630	979	67	5490	0
	sd			(1810)	(4960)	(794)	(24)	(4640)	
7. 2-Pentenal	mn	599	0	107	60	169	0	0	0
	sd	(279)		(58)	(49)	(32)			
8. 2-Hexenal	mn	1890	0	214	272	2190	0	116	0
	sd	(439)		(125)	(158)	(277)		(64)	
9. 2-Heptenal	mn	3700	0	920	496	36300	374	951	0
	sd	(448)		(379)	(40)	(13800)	(233)	(153)	
10. 2-Octenal	mn	3000	0	1460	0	31800	0	2620	0
	sd	(206)		(466)		(14100)		(317)	
11. 2-Nonenal	mn	4580	238	1060	1440	13400	1650	171	0
	sd	(706)	(95)	(459)	(475)	(2150)	(826)	(190)	
12. 2-Decenal	mn	9240	609	570	425	32300	370	214	0
	sd	(1560)	(135)	(39)	(134)	(10900)	(321)	(173)	
13. 2-Undecenal	mn	5440	890	259	292	19000	102	144	0
	sd	(291)	(180)	(66)	(180)	(6440)	(65)	(173)	
14. 2,4-Hepta-dienal	mn	795	0	203	59	931	0	0	0
	sd	(144)		(65)	(20)	(143)			
15. 2,4-Octadienal	mn	135	0	207	0	252	0	0	0
	sd	(24)		(51)		(9)			
16. 2,4-Nonadienal	mn	203	0	0	27	719	0	4	0
	sd	(91)			(48)	(117)		(7)	

No. Compound	Lipid: Cysteine + ribose:	BTG -	BTG +	BPL -	BPL +	PC -	PC +	PE -	PE +
17. tr,cis/cis,tr	mn	297	0	266	105	26800	124	161	0
2,4-Decadienal	sd	(39)		(59)	(7)	(6760)	(127)	(79)	
18. tr,tr-2,4-	mn	839	184	815	478	26400	1330	676	78
Decadienal	sd	(185)	(8)	(369)	(34)	(3360)	(560)	(288)	(40)
19. 2,4-Undeca-	mn	65	0	6	0	321	0	0	0
dienal	sd	(14)		(6)		(65)			
20. 2-Hexanone	mn	307	162	268	436	208	128	485	201
	sd	(47)	(19)	(100)	(244)	(71)	(45)	(173)	(70)
21. 2-Heptanone	mn	974	466	7570	10500	2900	5930	51400	34900
	sd	(144)	(220)	(1310)	(1060)	(364)	(2650)	(19200)	(2110)
22. 2-Octanone	mn	148	74	3410	4830	1000	2680	8240	7040
	sd	(39)	(61)	(423)	(1190)	(163)	(1180)	(3060)	(2130)
23. 2-Nonanone	mn	271	54	5720	3650	417	985	13600	11400
	sd	(171)	(93)	(957)	(364)	(25)	(678)	(4210)	(911)
24. 2-Decanone	mn	65	0	14400	8500	486	788	30000	19300
	sd	(28)		(1570)	(976)	(107)	(378)	(17900)	(3590)
25. 3-Octanone	mn	46	194	449	754	2160	5450	1950	3030
	sd	(4)	(152)	(182)	(159)	(493)	(1770)	(1230)	(1560)
26. 4-Methyl-	mn	0	0	0	0	24300	22500	224	289
2-pentanone	sd					(1060)	(6090)	(77)	(278)
27. 4-Methylpent-	mn	2380	1320	592	568	1560	656	48700	51600
3-en-2-one	sd	(851)	(941)	(83)	(219)	(381)	(91)	(6720)	(22600)
28. 1-Hexanol	mn	817	870	336	1353	641	7490	656	4890
	sd	(260)	(144)	(225)	(105)	(153)	(3510)	(157)	(967)
29. 1-Heptanol	mn	2290	2180	721	676	3770	12800	2680	2000
	sd	(682)	(489)	(586)	(111)	(428)	(5170)	(1140)	(1780)
30. 1-Octanol	mn	1970	2330	1260	1580	4830	19500	3300	2600
	sd	(386)	(528)	(701)	(251)	(440)	(8770)	(1580)	(1690)
31. 1-Nonanol	mn	192	519	369	926	358	6750	246	737
	sd	(44)	(226)	(39)	(166)	(130)	(3350)	(209)	(338)
32. 1-Octen-3-ol	mn	340	213	6830	6660	25500	40600	30700	34500
	sd	(87)	(99)	(3320)	(1280)	(5260)	(21000)	(7960)	(18600)
33. trans-	mn	0	0	898	456	4980	7630	8830	7180
2-Octen-1-ol	sd			(838)	(126)	(999)	(2900)	(4550)	(4600)
34. cis-	mn	0	0	713	321	956	1800	1960	1540
2-Octen-1-ol	sd			(290)	(16)	(708)	(713)	(1120)	(1420)

No.	Compound	Lipid: / Cysteine + ribose:	BTG -	BTG +	BPL -	BPL +	PC -	PC +	PE -	PE +
35.	2-Butylfuran	mn	0	49	321	217	171	711	1120	569
		sd		(66)	(156)	(31)	(57)	(556)	(196)	(228)
36.	2-Pentylfuran	mn	387	1250	25500	21400	15600	34500	32200	50000
		sd	(170)	(1270)	(2810)	(5100)	(2810)	(4810)	(1900)	(22900)
37.	2-Hexylfuran	mn	59	153	1010	955	113	476	163	203
		sd	(10)	(116)	(224)	(78)	(46)	(111)	(49)	(82)
38.	2-Heptylfuran	mn	28	63	229	139	92	1820	239	411
		sd	(10)	(58)	(72)	(85)	(12)	(975)	(173)	(224)
39.	2-Octylfuran	mn	35	73	2060	1820	164	2110	645	906
		sd	(4)	(76)	(997)	(1010)	(76)	(1400)	(602)	(729)
	Total RPA[c]	mn	126	301	334	493	586	606	601	741
	($x 10^{-3}$)	sd	(6)	(34)	(37)	(51)	(45)	(245)	(178)	(201)

[a] Relative peak areas are expressed in terms of the mean and (standard deviation) relative to the peak area given by 1ng 1,2-dichlorobenzene = 100; values greater than 1000 are stated to three significant figures.

[b] The four lipids were: beef triglyceride (BTG), beef phospholipid (BPL), egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE).

[c] Total RPA: total ion area of peaks eluting between 150 and 2500 seconds, relative to 1ng 1,2-dichlorobenzene = 100

Table 3.3b: Quantities[a] (ng) of selected compounds collected from the headspace from lipids heated in the absence and presence of cysteine and ribose.

	Lipid[b]:	BTG		BPL		PC		PE		no lipid	
	Cysteine + ribose:	-	+	-	+	-	+	-	+	-	+
No. Compound											
1. Hexanal	mn	208	97.6	551	276	516	428	1320	428	16.5	4.5
	sd	(1.9)	(46.7)	(225)	(42.0)	(70.6)	(146)	(219)	(109)	(2.1)	(3.5)
2. Heptanal	mn	229	89.7	375	171	333	353	1110	213	14.4	1.1
	sd	(22.0)	(23.6)	(98.5)	(21.9)	(6.4)	(125)	(226)	(151)	(1.1)	(2.3)
3. Octanal	mn	100	6.4	231	14.1	202	78.5	488	21.2	14.4	0
	sd	(12.2)	(1.7)	(95.8)	(5.6)	(8.4)	(46.7)	(153)	(19.9)	(5.9)	
4. Nonanal	mn	125	33.3	204	42.3	377	185	286	22.0	20.5	5.6
	sd	(12.6)	(12.0)	(68.8)	(9.0)	(128)	(31.6)	(57.5)	(4.7)	(8.3)	(4.2)
5. Decanal	mn	10.5	3.4	20.0	10.8	66.2	15.4	31.7	6.5	5.3	0.4
	sd	(4.0)	(1.1)	(7.9)	(7.7)	(23.8)	(4.7)	(12.7)	(3.6)	(3.1)	(0.9)
6. Hexadecanal[c]	mn	-	-	-	-	-	-	-	-	-	-
	sd										
7. 2-Pentenal	mn	21.6	0	3.9	2.2	6.1	0	0	0	0	0
	sd	(10.1)		(2.1)	(1.8)	(1.1)					
8. 2-Hexenal	mn	24.4	0	2.8	3.5	28.2	0	1.5	0	0	0
	sd	(5.7)		(1.6)	(2.0)	(3.6)		(0.8)			
9. 2-Heptenal	mn	79.3	0	19.7	10.6	779	8.0	20.4	0	0.3	0
	sd	(9.6)		(8.1)	(0.8)	(297)	(5.0)	(3.3)		(0.5)	
10. 2-Octenal	mn	38.7	0	18.9	0	410	0	33.8	0	0	0
	sd	(2.7)		(6.0)		(182)		(4.1)			
11. 2-Nonenal	mn	137	7.1	31.7	42.8	399	49.3	5.1	0	0.8	0
	sd	(21.1)	(2.8)	(13.7)	(14.2)	(64.1)	(24.6)	(5.7)		(1.1)	
12. 2-Decenal	mn	202	13.3	12.5	9.3	707	8.1	4.7	0	5.5	0
	sd	(34.1)	(3.0)	(0.9)	(2.9)	(238)	(7.0)	(3.8)		(7.8)	
13. 2-Undecenal	mn	78.3	12.8	3.7	4.2	273	1.5	2.1	0	1.1	0.3
	sd	(4.2)	(2.6)	(1.0)	(2.6)	(92.6)	(0.9)	(2.5)		(1.5)	(0.5)
14. 2,4-Hepta-dienal	mn	13.7	0	3.5	1.0	16.0	0	0	0	0	0
	sd	(2.5)		(1.1)	(0.3)	(2.5)					
15. 2,4-Octadienal	mn	2.2	0	3.4	0	4.1	0	0	0	0	0
	sd	(0.4)		(0.8)		(0.1)					
16. 2,4-Nonadienal	mn	4.0	0	0	0.5	14.2	0	0.1	0	0	0
	sd	(1.8)			(0.9)	(2.3)		(0.1)			

No. Compound		Lipid: BTG -	+	BPL -	+	PC -	+	PE -	+	no lipid -	+
17. tr,cis/cis,tr 2,4-Decadienal	mn	3.9	0	3.5	1.4	355	1.6	2.1	0	0	0
	sd	(0.5)		(0.8)	(0.1)	(89.5)	(1.7)	(1.1)			
18. tr,tr-2,4-Decadienal	mn	18.8	4.1	18.3	10.7	592	29.7	15.1	1.7	0	0
	sd	(4.1)	(0.2)	(8.3)	(0.8)	(75.3)	(12.6)	(6.4)	(0.9)		
19. 2,4-Undecadienal	mn	1.3	0	0.1	0	6.6	0	0	0	0	0
	sd	(0.3)		(0.1)		(1.3)					
20. 2-Hexanone	mn	4.0	2.1	3.4	5.6	2.7	1.7	6.2	2.6	1.3	1.7
	sd	(0.6)	(0.2)	(1.3)	(3.1)	(0.9)	(0.6)	(2.2)	(0.9)	(0.6)	(1.2)
21. 2-Heptanone	mn	7.6	3.6	58.8	81.9	22.5	46.1	399	271	0	0.06
	sd	(1.1)	(1.7)	(10.1)	(8.3)	(2.8)	(20.6)	(149)	(16.4)		(0.12)
22. 2-Octanone	mn	1.2	0.6	27.2	38.5	8.0	21.4	65.7	56.1	0	0.03
	sd	(0.3)	(0.5)	(3.4)	(9.5)	(1.3)	(9.4)	(24.4)	(17.0)		(0.06)
23. 2-Nonanone	mn	1.9	0.4	39.3	25.1	2.9	6.8	93.2	78.4	0	0
	sd	(1.2)	(0.6)	(6.6)	(2.5)	(0.2)	(4.7)	(28.9)	(6.3)		
24. 2-Decanone	mn	0.5	0	108	63.8	3.7	5.9	225	145	0	0
	sd	(0.2)		(11.8)	(7.3)	(0.8)	(2.8)	(134)	(26.9)		
25. 3-Octanone	mn	0.4	1.9	4.4	7.4	21.0	53.1	18.9	29.6	0.2	0
	sd	(0.04)	(1.5)	(1.8)	(1.6)	(4.8)	(17.3)	(12.0)	(15.2)	(0.1)	
26. 4-Methyl-2-pentanone	mn	0	0	0	0	1110	1020	10.2	13.2	0	0
	sd					(48.2)	(277)	(3.5)	(12.7)		
27. 4-Methylpent-3-en-2-one	mn	32.8	18.3	8.2	7.8	21.5	9.1	672	712	4.9	1.6
	sd	(11.8)	(13.0)	(1.2)	(3.0)	(5.3)	(1.3)	(92.7)	(312)	(2.7)	(1.6)
28. 1-Hexanol	mn	8.6	9.1	3.5	14.2	6.7	78.6	6.9	51.3	0.3	0.2
	sd	(2.7)	(1.5)	(2.4)	(1.1)	(1.6)	(36.9)	(1.7)	(10.1)	(0.1)	(0.2)
29. 1-Heptanol	mn	23.0	21.9	7.3	6.8	37.9	128	27.0	20.1	0.6	0.6
	sd	(6.9)	(4.9)	(5.9)	(1.1)	(4.3)	(52.0)	(11.5)	(17.9)	(0.04)	(0.5)
30. 1-Octanol	mn	16.4	19.4	10.5	13.1	40.1	162	27.4	21.6	2.6	3.3
	sd	(3.2)	(4.4)	(5.8)	(2.1)	(3.7)	(72.9)	(13.1)	(14.0)	(0.3)	(1.1)
31. 1-Nonanol	mn	1.6	4.3	3.1	7.7	3.0	55.7	2.0	6.1	1.5	2.9
	sd	(0.4)	(1.9)	(0.3)	(1.4)	(1.1)	(27.7)	(1.7)	(2.8)	(1.0)	(0.7)
32. 1-Octen-3-ol	mn	4.5	2.8	90.0	87.8	336	536	405	455	0.2	0.2
	sd	(1.1)	(1.3)	(43.7)	(16.9)	(69.4)	(277)	(105)	(245)	(0.3)	(0.4)
33. trans-2-Octen-1-ol	mn	0	0	9.5	4.8	52.8	80.9	93.5	76.1	0	0
	sd			(8.9)	(1.3)	(10.6)	(30.8)	(48.2)	(48.7)		
34. cis-2-Octen-1-ol	mn	0	0	8.1	3.6	10.8	20.4	22.2	17.4	0	0
	sd			(3.3)	(0.2)	(8.0)	(8.1)	(12.6)	(16.0)		

Cysteine + ribose: - / + under each lipid.

No.	Compound	Lipid: Cysteine + ribose:	BTG -	BTG +	BPL -	BPL +	PC -	PC +	PE -	PE +	no lipid -	no lipid +
35.	2-Butylfuran	mn	0	0.7	3.5	2.4	1.9	7.8	12.3	6.2	0	0
		sd		(0.6)	(1.7)	(0.3)	(0.6)	(6.1)	(2.1)	(2.5)		
36.	2-Pentylfuran	mn	3.7	11.9	243	205	149	329	307	477	0	0
		sd	(1.6)	(12.1)	(26.8)	(48.7)	(26.8)	(45.9)	(18.1)	(218)		
37.	2-Hexylfuran	mn	0.5	1.3	8.7	8.2	1.0	4.1	1.4	1.7	0	0
		sd	(0.1)	(1.0)	(1.9)	(0.7)	(0.4)	(1.0)	(0.4)	(0.7)		
38.	2-Heptylfuran	mn	0.2	0.5	2.0	1.2	0.8	15.6	2.0	3.5	0	0
		sd	(0.1)	(0.5)	(0.6)	(0.7)	(0.1)	(8.3)	(1.5)	(1.9)		
39.	2-Octylfuran	mn	0.3	0.5	15.0	13.3	1.2	15.4	4.7	6.6	0	0
		sd	(0.03)	(0.6)	(7.3)	(7.4)	(0.6)	(10.2)	(4.4)	(5.3)		

[a] Quantities (ng) are expressed in terms of the mean and (standard deviation) to three significant figures or one decimal place.

[b] The four lipids were: beef triglyceride (BTG), beef phospholipid (BPL), egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE).

[c] Authentic material not available in sufficient quantities.

Table 3.3c: Odour descriptions for heated reaction mixtures containing lipids alone.

Reaction mixture		Odour descriptions
2	Buffer blank	Odourless, watery
4	Beef triglyceride	Fatty, very weak, greasy, no species-specific aroma
6	Beef phospholipid	Chicken, meaty, beef dripping, fatty
8	Egg PC	Fatty, chicken, chicken skin
10	Egg PE	Good chicken aroma, chicken soup/broth/pies/skin

chloroform-methanol extracts from pork, beef and lamb possess aromas characteristic of these species, washing the extracts with water removed the components required for the formation of characteristic odours. Thus, although early studies on the formation of meat flavour suggested that the species-specific aromas of meats may be developed in the fat, it was concluded that the formation of the compounds responsible required the presence of the aqueous cell components (Wasserman and Spinelli 1972). On the other hand, the species-characteristic odour of lamb and mutton which is beleived to be caused by 4-methyloctanoic and 4-methylnonanoic acids, derived from the oxidation of branched-chain fatty acids (Wong *et al* 1975).

The odours of the three phospholipids were very different from that of beef triglyceride. Heated PE gave a particularly good "chicken" aroma, with beef phospholipid and PC also possessing chicken-like notes.

Phospholipids have been held reponsible for the development of rancidity in poultry meat (Ramaswamy and Richards 1982), but might also be associated with pleasant cooked chicken aroma. Such a possibility seems inconsistent with the results of Koehler and Jacobson (1967), who examined water extracts of minced chicken and concluded that the constituents of chicken muscle responsible for flavour are water-soluble and dialyzable. More recent studies of the individual odour components of 'dripping fat' from roast

chicken established the presence of compounds possessing a chicken-like odour but did not identify them (Noleau and Toulemonde 1987). However, chicken volatiles are characterized by a series of unusual alkadienals and alkatrienals, believed to arise from the breakdown of arachidonic acid and it is suggested that such aldehydes contribute to chicken flavour (Harkes and Begemann 1974). Thus, the pronounced chicken-like aroma derived from PE, heated in the absence of other reactants, may arise from the particularly high levels of arachidonic acid in this phospholipid.

3.3.2 VOLATILES FROM THE DEGRADATION OF LIPIDS HEATED ALONE

Before an evaluation is made of the effect of cysteine and ribose on the volatile products of lipid oxidation, it is worthwhile commenting on the products obtained from each lipid when heated alone. A direct comparison cannot be made between the absolute amounts of volatile products yielded by the four lipids, because the overall concentration of fatty acids differed between the lipids. As it would have been impossible to match both the content of fatty acids and of choline and ethanolamine groups, the same overall mass of lipid was used in all cases. This meant that the phospholipids had a lower content of fatty acids (*ca* 70%) than the triglyceride (90%).

In order to compare gross differences between the volatiles obtained from each lipid, the following method was used to estimate the contribution made by each compound class to the total eluted volatiles. The 'total RPA' for each system was obtained by expressing the total ion area for all eluted peaks relative to the internal standard, and is a measure of the total area under the chromatogram (Table 3.3a). The sum of the RPAs for each compound class can be stated as a percentage of the 'total RPA', and thus represents the contribution made by each compound class to the total peak area (Table 3.3d). This method does not take into account different response factors for each compound, but provides a simple (albeit imprecise) means for comparing the contribution

Table 3.3d: Contribution of compound classes to the headspace volatiles from each lipid, as percentage of total RPA[a]

Compound class	Compound numbers	Lipid[b] BTG	BPL	PC	PE
Saturated n-aldehydes	1-6	26.7	24.5	14.4	24.9
2-Alkenals	7-13	22.6	1.4	23.1	0.7
2,4-Alkadienals	14-19	1.9	0.4	9.5	0.1
2-Alkanones	20-24	1.4	9.4	0.9	17.3
Misc. ketones	25-27	1.9	0.3	4.8	8.5
1-Alcohols	28-31	4.2	0.8	1.6	1.2
Unsat. alcohols	33-34	0.3	2.5	5.4	6.9
2-Alkylfurans	35-39	0.4	8.7	2.7	5.7
Percentage of total RPA accounted for:		59.3	48.1	62.3	65.3

[a] The sum of the RPAs (relative peak areas) for the members of each compound class was expressed as a percentage of the total RPA (Table 3.3a).

[b] BTG - beef triglyceride; BPL - beef phospholipid; PC - egg phosphatidylcholine; PE - egg phosphatidylethanolamine

made by the various compound classes to the headspace from each of the four lipids.

A comparison of the values obtained for the total RPA for the four lipids (given in Table 3.3a) indicates that the triglyceride produces less than half the 'area' of volatile products than any of the phospholipids. This is to be expected from the lower proportion of polyunsaturated fatty acids present in the triglyceride. As was discussed in Section 1.2.1.1 the energy required to initiate free radical oxidation decreases with increasing degree of unsaturation, resulting in a much higher rate of formation of peroxides and consequently of volatile breakdown products.

In addition, the total RPA from beef phospholipid is lower than from the two egg phospholipids; this observation is less readily

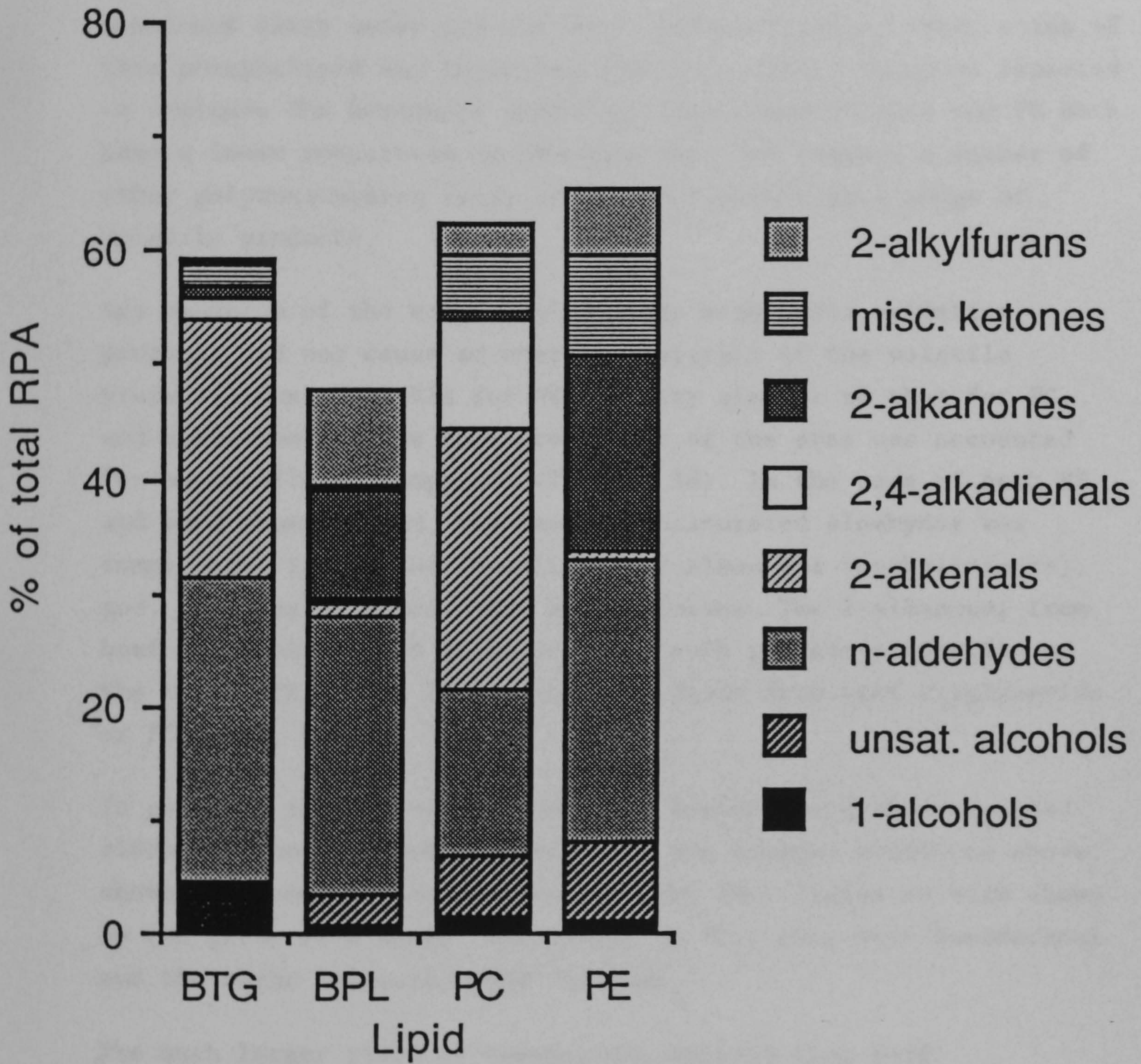
explained as beef phospholipid contains the highest proportion of polyunsaturated fatty acids.

The contribution made by each compound class to the total RPA showed some pronounced differences between the four lipids (Table 3.3d). These differences are shown diagramatically in Figure 3.3A.

Approximately 50% of the total peak area of volatiles derived from beef triglyceride and PC was due to aldehydes. For beef phospholipid and PE the proportion of aldehydes was only half this value. This contrast can be attributed almost entirely to differences in the proportions of unsaturated aldehydes. Although saturated aldehydes comprised approx. 25% of the chromatogram area for all the lipids (except PC: 14.4%), the levels of 2-alkenals and 2,4-alkadienals derived from beef phospholipid and PE were more than 10 times lower than from beef triglyceride and PC. Beef phospholipid and PE are both rich in highly unsaturated fatty acids which should be particularly susceptible to oxidation and might be expected to yield unsaturated aldehydes by the pathways illustrated in Figures 1.2H and J (Sec. 1.2.1.2). However, both these lipids contain the ethanolamine polar group and it is likely that the unsaturated aldehydes are removed from the system by reaction with this amine. The reaction of lipid-derived aldehydes with the amino groups of ethanolamine and also protein amino groups has already been described (Sections 1.2.3 and 1.3.1.1). Such reactions result in the formation of Schiff base intermediates and thence polymeric browning products, formed by condensation reactions. There is evidence that unsaturated aldehydes are more reactive with ethanolamine than their saturated counterparts (Fujimoto *et al* 1968) and this would accord with the above results. It is surprising, however, that the saturated aldehydes were apparently unaffected by the presence of the ethanolamine group. In the absence of the more labile unsaturated compounds, the reaction of saturated aldehydes may occur more readily.

The aldehydes showing the largest difference between PC and the other phospholipids are the C_7-C_{11} 2-alkenals and 2,4-decadienal. The hypothesis that these aldehydes may undergo reaction with the ethanolamine of beef phospholipid and PE is supported by the fact that the volatiles from beef triglyceride contain higher levels of alkadienals than these two phospholipids, despite the paucity of

Figure 3.3A: Contribution of volatile compound classes, obtained from lipids heated alone, to total area under the chromatogram



polyunsaturated fatty acids in this lipid; triglyceride contains only low levels of linoleic acid and linolenic acid, yet these fatty acids are probably the origin of the 2,4-decadienal and 2,4-heptadienal observed. 2,4-Decadienal is a predicted breakdown product of the 9-hydroperoxide of linoleic acid and also the 11-hydroperoxide of arachidonic acid. The extremely high levels of this compound collected from the heated PC, compared with the other lipids, may also be related to the fact that the above-mentioned fatty acids are the major polyunsaturated fatty acids of this phospholipid and their degradation products would be expected to dominate the headspace volatiles; beef phospholipid and PE both have a lower proportion of linoleic acid and contain a number of other polyunsaturated fatty acids, each producing a range of volatile products.

Any reaction of the ethanolamine group with lipid oxidation products did not cause an overall depletion of the volatile products; the total RPA for PE was very similar to that for PC, and approximately the same proportion of the area was accounted for by the listed compounds (Table 3.3d). In the case of both PE and beef phospholipid, the lack of unsaturated aldehydes was compensated for by the formation of 2-alkanones (methylketones), and, to a lesser extent, the 2-alkylfurans. The 2-alkanones from beef phospholipid and PE comprised a much greater proportion of the total RPA (9.4%, 17.3%) than did those from beef triglyceride or PC (1.4%, 0.9%).

In general, the individual compounds listed among the saturated aldehydes, unsaturated aldehydes and the ketones mentioned above, showed the same differences between the four lipids as were shown by the group as a whole. Exceptions to this rule were hexadecanal and the three 'miscellaneous' ketones.

The much larger yield of hexadecanal derived from beef phospholipid compared with the other lipids is almost certainly caused by the high plasmalogen content of this phospholipid. Plasmalogens possess an ether linkage at the *sn1* position instead of an ester linkage to a fatty acid (Christie 1973); a number of long-chain aldehydes, including hexadecanal, were detected among the fatty acids during analysis by GC-MS (see Table 3.2a). It seems likely that this aldehyde was formed directly via cleavage of the plasmalogen linkage rather than by oxidation pathways.

The proportions of the 'miscellaneous ketones' differed between the four lipids due to variations in the behaviour of the individual compounds. 4-Methyl-2-pentanone was produced in large quantities only by PC, unlike 4-methylpent-3-en-2-one which was formed in considerable amounts by PE and, to a lesser extent, beef triglyceride. More 3-octanone was produced by the two egg phospholipids than by either beef triglyceride or phospholipid. The reason for these differences is not apparent.

The overall proportion of alcohols was similar for all the lipids; however, beef triglyceride gave mainly saturated 1-alcohols and little of the unsaturated octenols, while for the three phospholipids the converse was true. This would appear to reflect the levels of polyunsaturated fatty acids in these lipids as 1-octen-3-ol and 2-octen-1-ol are both predicted breakdown products of such fatty acids (Wilkinson and Stark 1967; Forss 1972).

Although the 2-alkylfurans comprised only a small proportion of the total area, their contribution was higher in the volatiles from BPL and PE. As these compounds are believed to be formed from hydroperoxides containing a diene system, this observation would seem to be in accord with the greater proportion of polyunsaturated fatty acids in these two phospholipids.

An alternative route of formation of alkylfurans is from the alkadienals (Nonaka *et al* 1967). However, lower levels of furans were detected in the volatiles from PC than from the other two phospholipids, despite the fact that by far the highest levels of alkadienals were detected in the volatiles from this phospholipid. This would seem to suggest that, in these systems, the 2-alkylfurans are formed from the hydroperoxides rather than from the 2,4-alkadienals.

3.3.3 EFFECT OF CYSTEINE + RIBOSE ON LIPID-DERIVED VOLATILES

The main purpose of this experiment was to examine the effect of the Maillard reactants on the thermal degradation products from each individual lipid. It is evident from the results shown in Tables 3.3a and b that the presence of Maillard reactants did not

affect all the aliphatic compounds in the same way; considerable differences were observed between compound classes. In particular, the aldehydes were considerably reduced by the presence of the Maillard reactants, while other classes such as ketones and alcohols were unaffected or were even increased. The possible reasons for these differences will be discussed for each compound class.

Aldehydes

The levels of all the aldehydes formed from each of the four lipids were reduced in the presence of cysteine and ribose, as illustrated in Figures 3.3B to D. While the saturated aldehydes were usually reduced by a factor of about two, the unsaturated aldehydes (2-alkenals and 2-alkadienals) were often reduced by factors of more than ten, or were removed from the headspace completely. Even PC, which produced the highest quantities of alkenals and alkadienals in the absence of cysteine and ribose, produced very little in their presence.

Trace amounts of some aliphatic compounds were detected among the volatiles from cysteine and ribose and from the buffer alone (Table 3.2b), despite careful conditioning of the Tenax traps and additional precautions to prevent cross-contamination (see Sec. 6). In general, the amounts of these compounds were extremely low compared with those derived from the lipid-containing systems and do not affect the results as a whole, but the C_8 to C_{10} aldehydes proved difficult to eliminate. The low levels of these compounds detected in some lipid/Maillard-containing systems may, therefore, be slightly inaccurate. However, the amounts of these aldehydes detected in the systems containing lipid alone were large and the effect of cysteine and ribose in reducing their levels is not in doubt.

The reduction in the levels of aldehydes in the presence of cysteine and ribose could be caused either by the suppression of the pathways responsible for their formation or by further reactions with other components. Various Maillard reaction intermediates and products may exert an antioxidative effect; such compounds probably react with free radicals, thus inhibiting the progression of oxidation (Sec. 1.3.2). However, the fact that

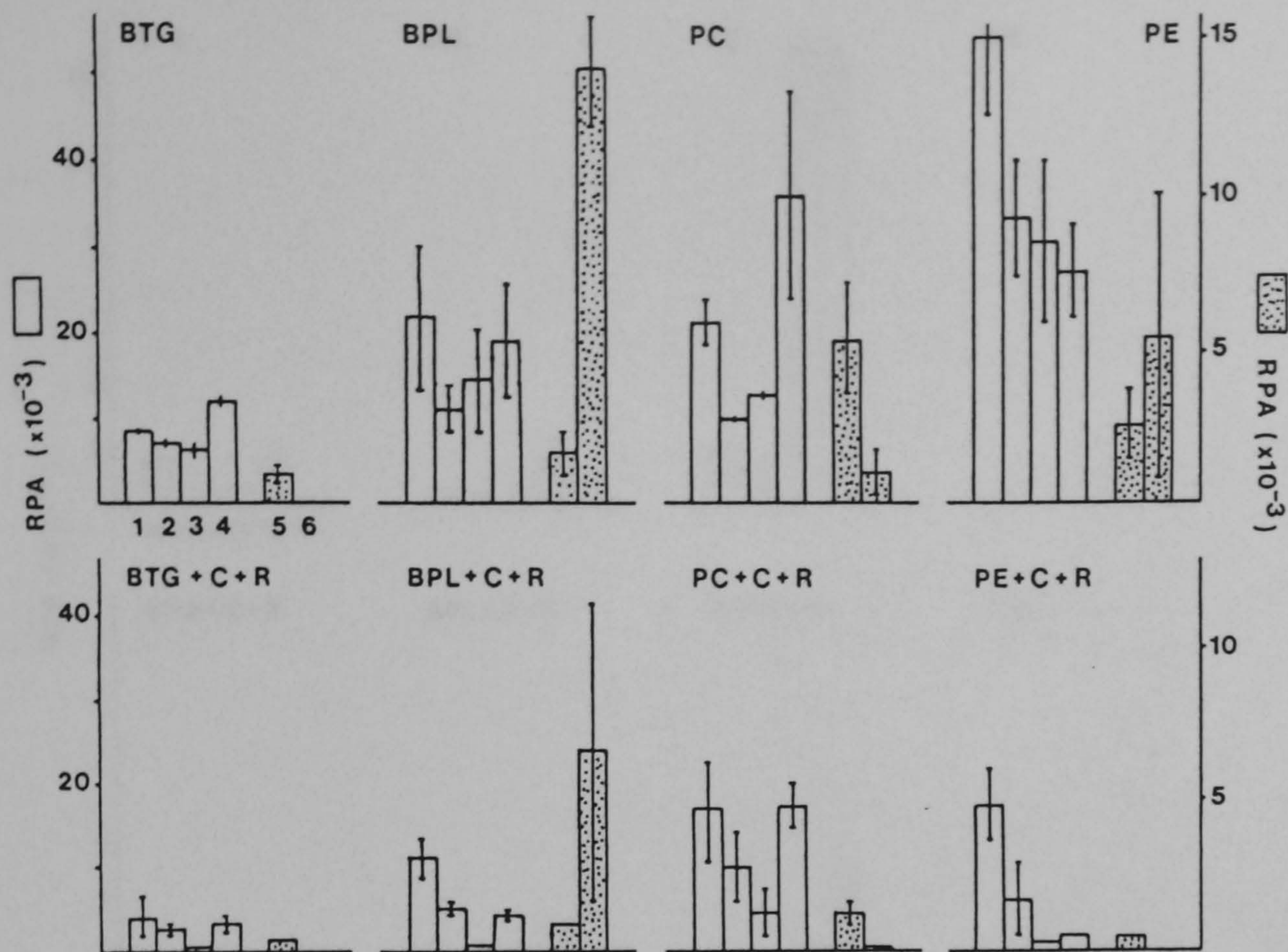


Figure 3.3B: Effect of cysteine and ribose on the formation of n-aldehydes from heated lipids.

1 Hexanal
2 Heptanal
3 Octanal
4 Nonanal
5 Decanal
6 Hexadecanal

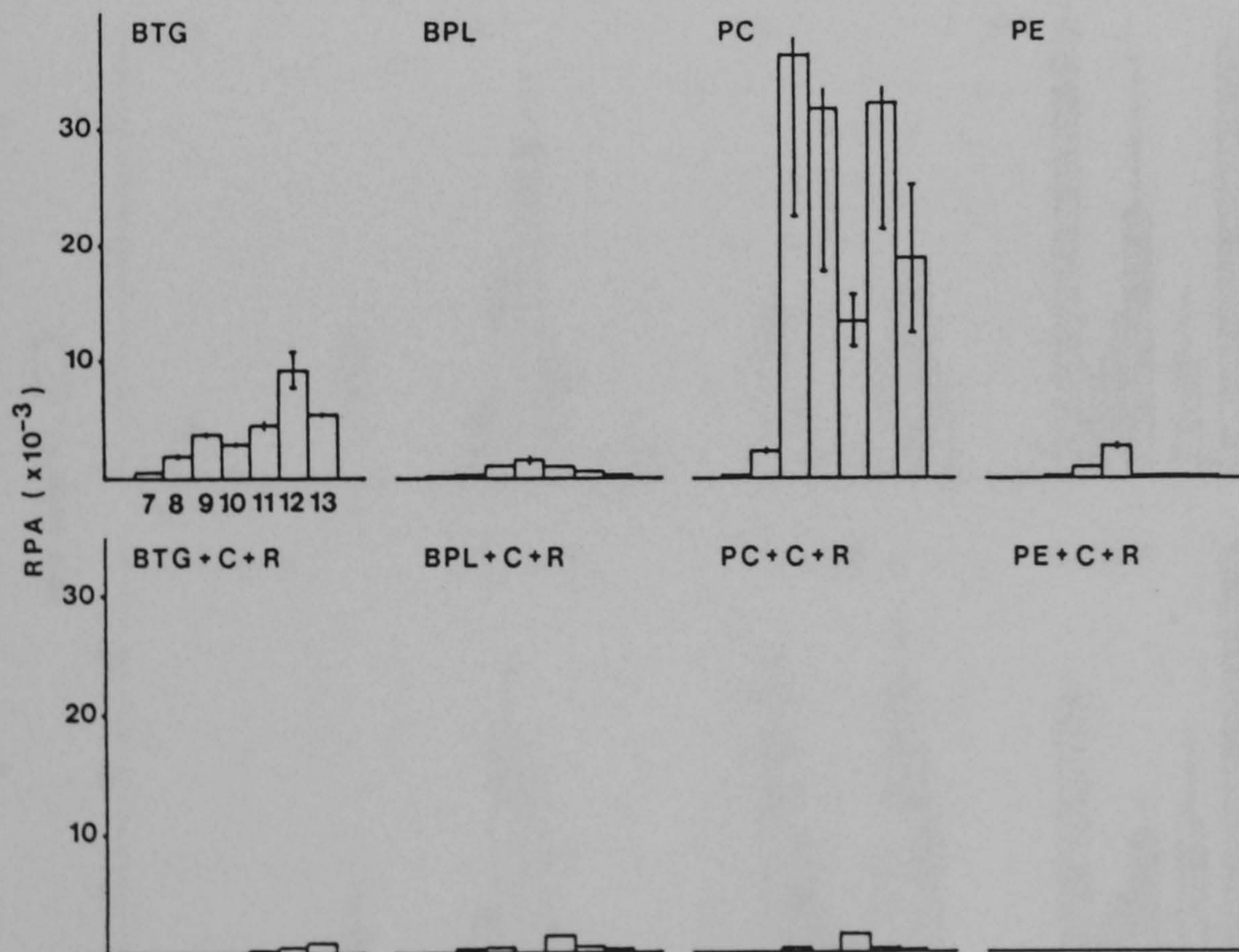


Figure 3.3C: Effect of cysteine and ribose on the formation of 2-alkenals from heated lipids.

7 2-Pentenal
8 2-Hexenal
9 2-Heptenal
10 2-Octenal
11 2-Nonenal
12 2-Decenal
13 2-Undecenal

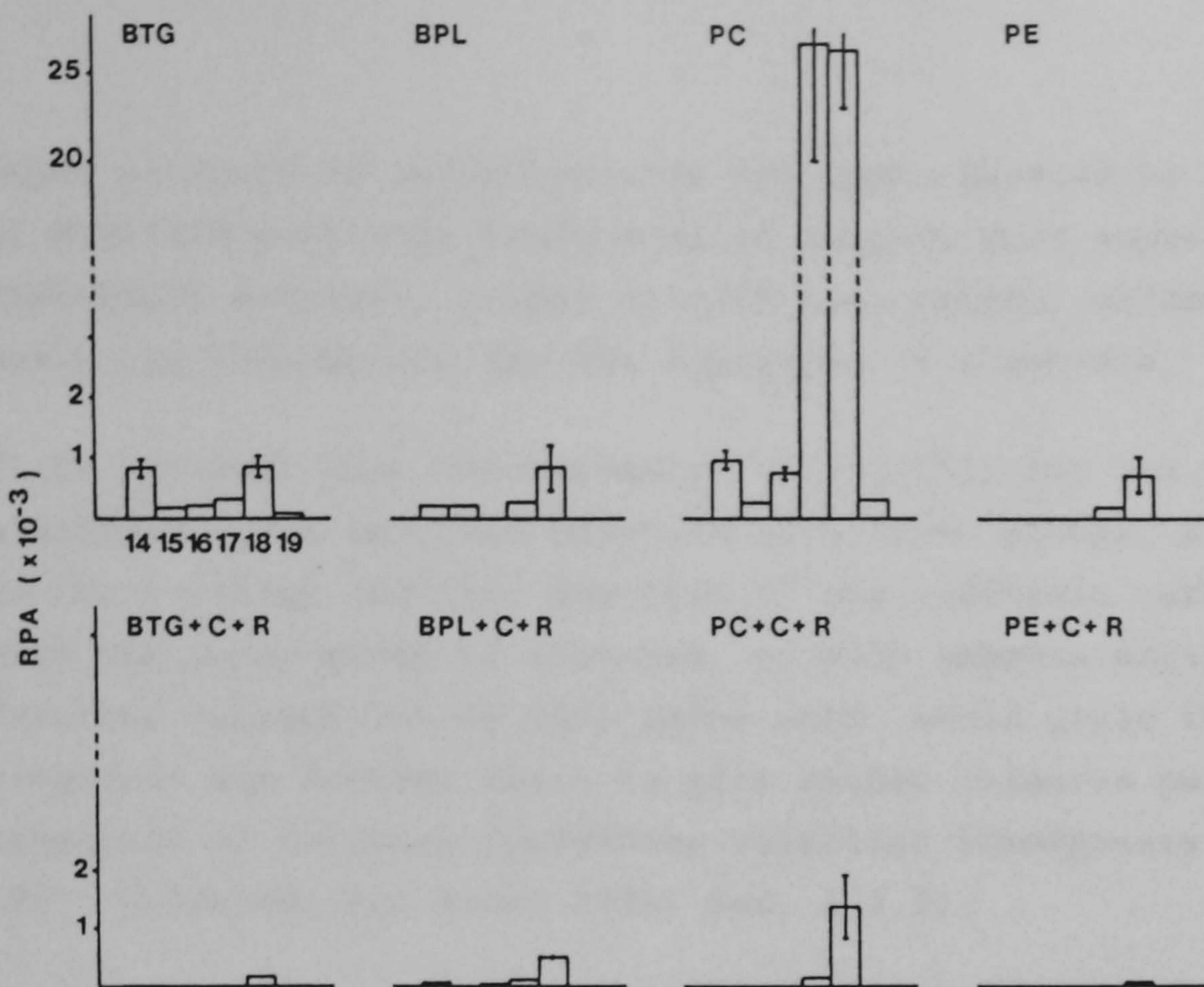


Figure 3.3D: Effect of cysteine and ribose on the formation of 2,4-alkadienals from heated lipids.

14	2,4-Heptadienal	17	2,4-Decadienal (*tr,cis/cis,tr*)
15	2,4-Octadienal	18	2,4-Decadienal (*tr,tr*)
16	2,4-Nonadienal	19	2,4-Undecadienal

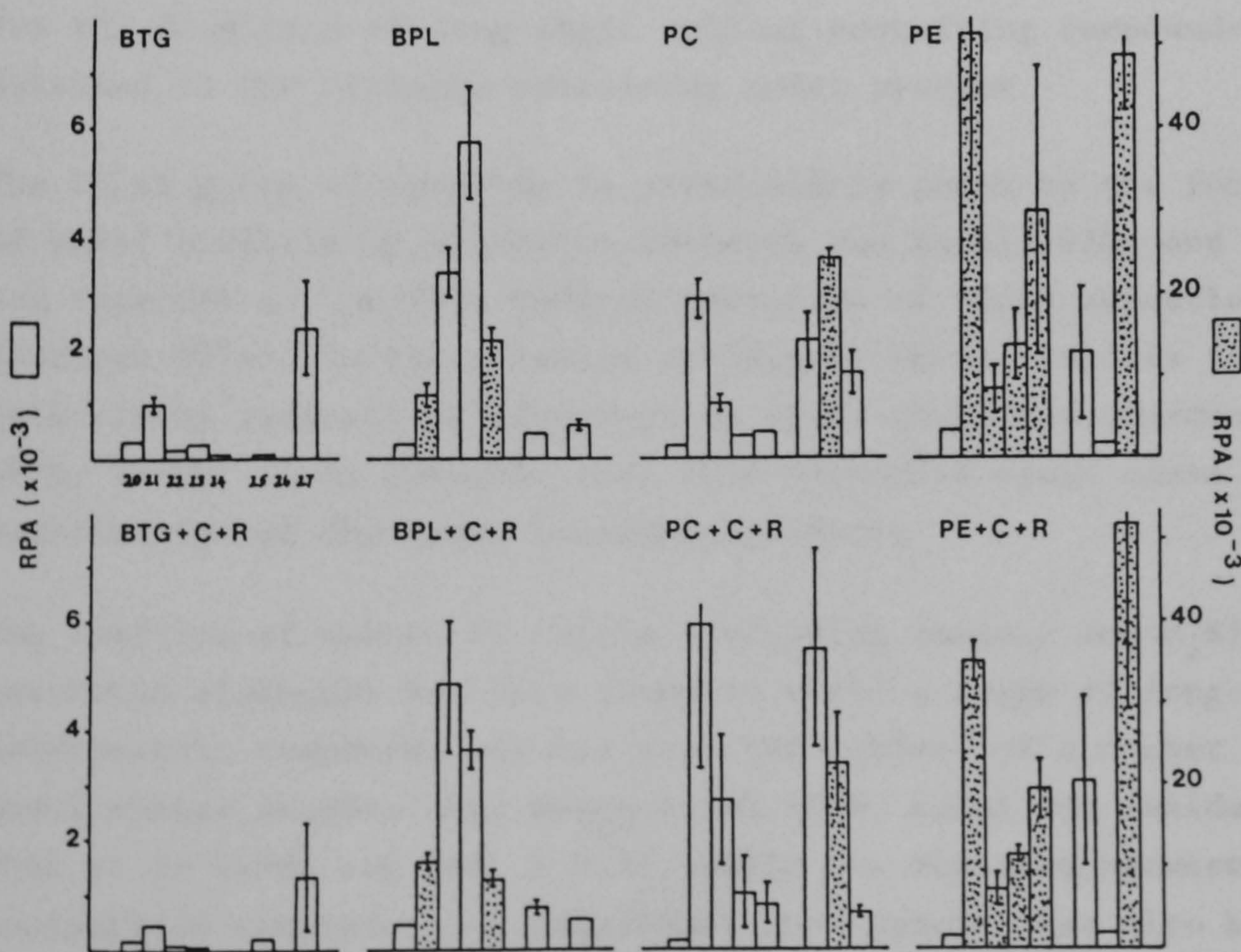


Figure 3.3E: Effect of cysteine and ribose on the formation of ketones from heated lipids.

20	2-Hexanone	23	2-Nonanone	25	3-Octanone
21	2-Heptanone	24	2-Decanone	26	4-Methyl-2-pentanone
22	2-Octanone			27	4-Methylpent-3-en-2-one

other products of oxidation were not much affected by the presence of Maillard reactants would seem to suggest that suppression of individual pathways, rather than of free radical oxidation as a whole, is responsible for the depletion of aldehydes.

It is probable that the mechanism responsible for the depletion of aldehydes again involves reaction with amino groups, as discussed in the previous Section. Reaction of the aldehydic carbonyl group with the amino group of cysteine, or with ammonia derived from the Strecker degradation of this amino acid, would yield imines. These compounds may further react to give either coloured polymeric compounds or nitrogen-containing volatiles (Montgomery and Day 1965; Henderson and Nawar 1981; Sec. 1.3.3).

Other mechanisms have been described for the interaction between the Maillard reaction and lipid oxidation and these may also play a part in the suppression of aldehydes. In addition to Schiff base type reactions, the thiol group of cysteine is capable of reaction with the enol form of α,β-unsaturated aldehydes, such as malonaldehyde (Buttkus 1969). The reaction of unsaturated aldehydes with either cysteine or H_2S may be partly responsible for the formation of long-chain sulphur-containing compounds detected in the cysteine-containing model systems.

The thiol group of cysteine is particularly prone to the formation of thiyl radicals by oxidation (Schaich and Karel 1976) and thus can interact in the free radical reactions of lipid oxidation (Gardner 1976). In the presence of oxygen, these radicals react with alkoxy radicals to give keto or epoxy compounds (Gardner 1976, 1979); it is possible that this mechanism could cause modification of the final breakdown products.

The reaction of amines or thiols (including ammonia and H_2S) with saturated aldehydes has been shown to yield a range of long-chain heterocyclic compounds and has been the subject of a number of model system studies (eg. Hwang *et al* 1986; Kawai and Ishida 1987; Chiu *et al* 1990; see Sec. 1.3.3), while the reaction between an unsaturated aldehyde, 2,4-decadienal and cysteine has also been studied (Zhang *et al* 1989). However, few details of the mechanisms of these reactions have been published. The detection of long chain pyridines and pyrroles in the reactions between glycine with ribose and phospholipid, and the additional formation of alkyl and

alkenylthiophenes, thiapyrans and alkanethiols in the presence of cysteine were described in Section 2. The formation of such compounds must account, in part, for the observed depletion of the aldehydes.

Ketones

Unlike the aldehydes, the amounts of 2-alkanones in the headspace volatiles were little affected by the presence of Maillard reactants (Fig. 3.3E). Likewise the 'miscellaneous ketones' showed no consistent effect from the addition of cysteine and ribose.

Few classes of ketones are formed directly from lipid hydroperoxides (Figs. 1.2G-J); instead, most arise as products of the further oxidation of the major products or from the thermal oxidation of saturated fatty acids (Mookherjee *et al* 1965; Mottram 1990; see Sec. 1.2.4). The fact that these compounds are unaffected by the presence of cysteine and ribose would seem to preclude any overall antioxidative effect.

Alcohols

Cysteine and ribose had little effect on the levels of 1-alcohols formed by BTG. The effect on alcohols produced by BPL and PE varied; 1-heptanol and 1-octanol were little changed while 1-hexanol and 1-nonanol were increased. However, the greatest effect was that observed for PC; all the 1-alcohols were increased by factors ranging from 3 to 20. The effect of cysteine and ribose on the alcohols produced by each lipid is shown in Figure 3.3F.

The fact that the amounts of 1-alcohols detected among the headspace volatiles is only affected by the Maillard reactants in certain instances is difficult to explain. In particular, PC and PE give comparable amounts of these alcohols in the absence of cysteine and ribose, but vastly different amounts in their presence.

The main difference between these two phospholipids when heated alone was the high amounts of unsaturated aldehydes formed by PC. It is possible that the presence of cysteine and ribose somehow

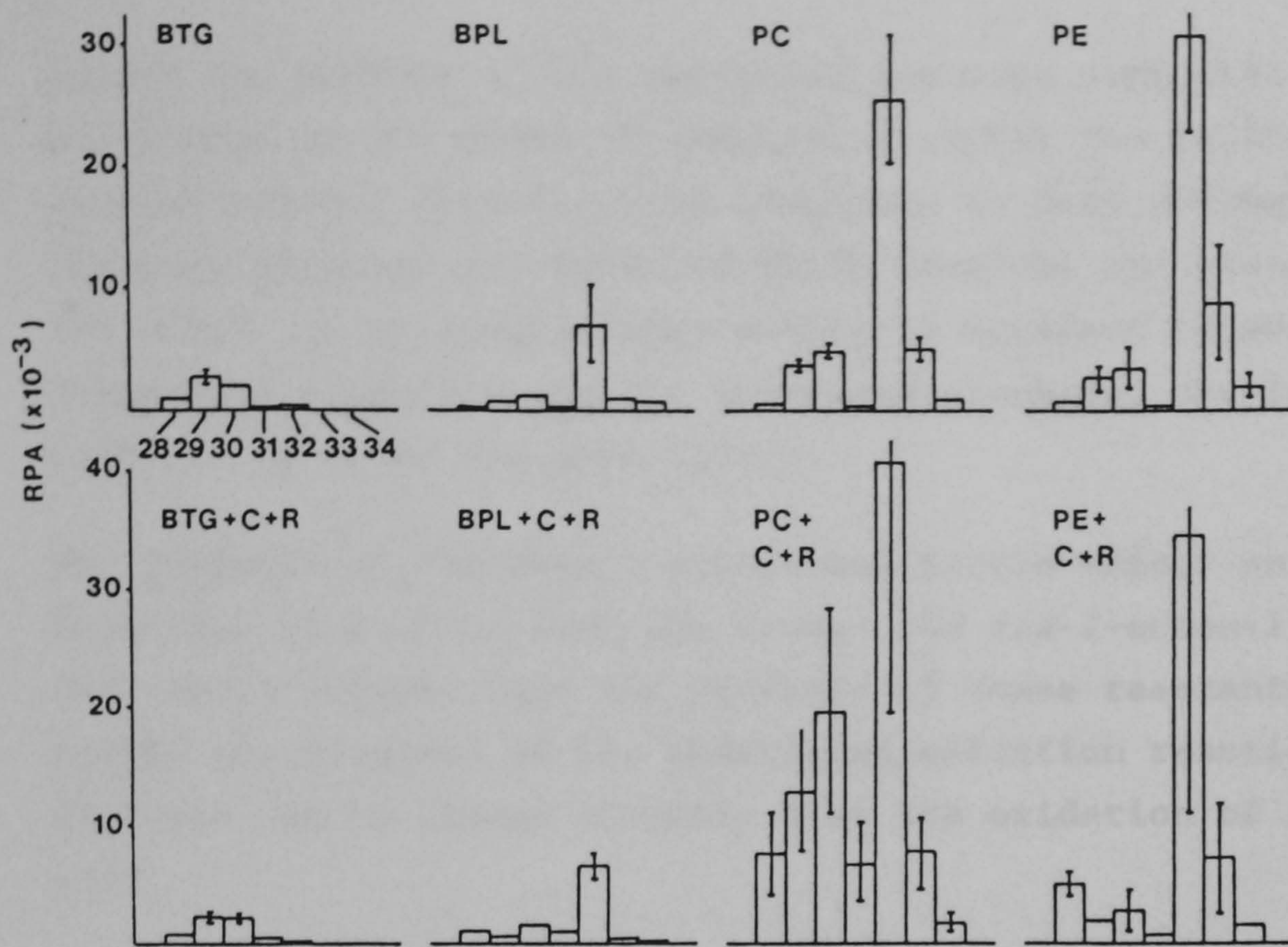


Figure 3.3F: Effect of cysteine and ribose on the formation of alcohols from heated lipids.

28 1-Hexanol
29 1-Heptanol
30 1-Octanol
31 1-Nonanol
32 1-Octen-3-ol
33 2-Octen-1-ol (*tr*)
34 2-Octen-1-ol (*cis*)

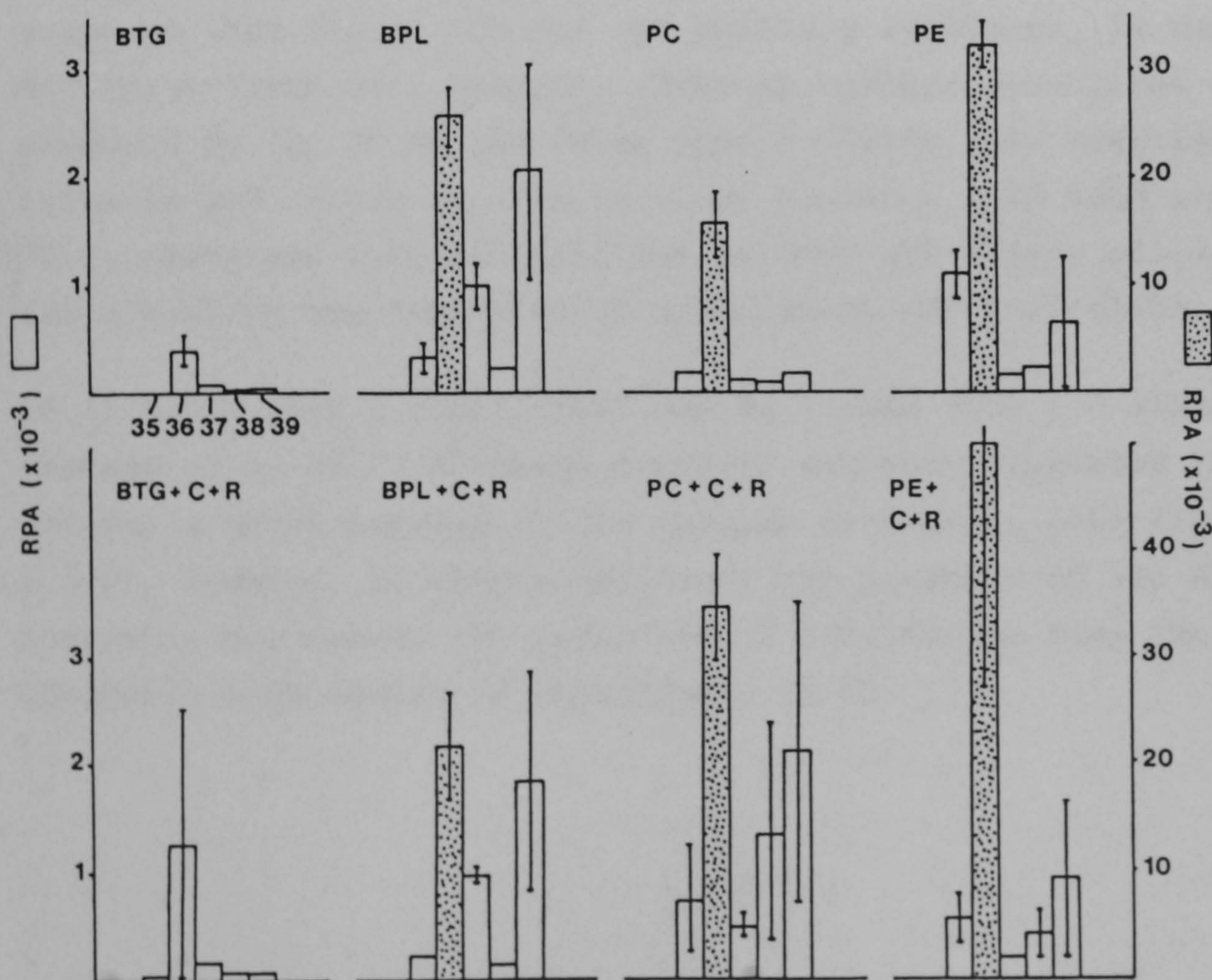


Figure 3.3G: Effect of cysteine and ribose on the formation of 2-alkylfurans from heated lipids.

35 2-Butylfuran
36 2-Pentylfuran
37 2-Hexylfuran
38 2-Heptylfuran
39 2-Octylfuran

alters the balance of the oxidation pathways such that alcohols are formed in the place of aldehydes; either the Maillard reactants react directly with aldehydes to form alcohols or reaction pathways are favoured which form the one class instead of the other. As no simple relationship is apparent between the diminished aldehydes and the increased alcohols, the latter possibility seems the more likely.

The addition of cysteine + ribose had little effect on the formation of 1-octen-3-ol and *trans*- and *cis*-2-octen-1-ol. Again, this would suggest that the presence of these reactants does not modify the progress of the underlying oxidation reactions as these alcohols can be formed directly from the oxidation of arachidonic acid.

Alkylfurans

The production of 2-alkylfurans appears to be unaffected by Maillard reactants except in the case of PC (Fig. 3.3G). This suggests that lipid, but not the Maillard reactants, is necessary for their formation. However, although similar quantities are produced by the PC as the other phospholipids, the addition of cysteine and ribose in this instance causes a 2-20 fold increase. Thus, there are some similarities between the effect of cysteine and ribose on the formation of alkylfurans and 1-alcohols.

It is known that 2-alkylfurans can be formed from 2,4-alkadienals (Nonaka *et al* 1967) although previous evidence suggested that this was not a major pathway in the systems containing only lipid (Sec. 3.3.2). However, it is possible that the presence of the Maillard reactants may favour the formation of alkylfurans from the unusually high levels of alkadienals in PC.

3.4 GENERAL DISCUSSION

The results described in Section 3.2 not only confirm that lipids interact in the Maillard reaction to modify the volatile aroma compounds produced, but also demonstrate marked dissimilarities between the behaviour of the four lipids. The most notable distinction was between the effect of triglyceride and the three phospholipids; with few exceptions those compounds whose mechanisms of formation were susceptible to lipid intervention were more affected by the presence of a phospholipid than by triglyceride.

The volatile compounds most affected by the presence of lipid were long-chain heterocyclic compounds requiring both Maillard reactants and lipids for their formation. In all cases, little or none of these compounds was formed in the presence of beef triglyceride, while maximum formation occurred in the Maillard system containing PC. Particularly abundant in this system were 2-pentylthiapyran and 2-pentylpyridine, whose formation from the reaction of 2,4-decadienal with H_2S or NH_3 may be partly responsible for the depletion of this aldehyde in presence of Maillard reactants (Sec. 3.3).

The mercaptoketones and the furan and thiophenethiols were considerably reduced by all the phospholipids, possibly due to a reduction in the concentration of available H_2S by reaction with unsaturated fatty acids or their oxidation products. Such reactions may also contribute to the depletion of aldehydes in the presence of cysteine and ribose. In addition to the clear difference between the triglyceride and the phospholipids, there were also variations in the way that the three phospholipids interacted in the Maillard reaction; the reduction in heterocyclic thiols was particularly marked in the systems containing beef phospholipid or PE. Both mercaptoketones and heterocyclic thiols can possess extremely low odour thresholds and the pronounced and distinct effects of the different classes of lipids on these compounds would be expected to influence the overall odour of cooked foods.

The contribution made by the products of lipid oxidation to the aroma volatiles is modified considerably by heating with cysteine

and ribose. The most pronounced alteration was the reduction of the amounts of aldehydes detected, especially the unsaturated aldehydes. These changes are consistent with the occurrence of reactions between the aldehydic carbonyl group and amino or thiol groups (including NH_3 and H_2S). The lower levels of unsaturated aldehydes formed by phospholipids containing ethanolamine as a polar moiety can probably be attributed to similar Schiff base type reactions. The depletion of aldehydes by Maillard reactants was greatest where the lipid did not itself contain ethanolamine amino groups. The increase in the detected levels of 1-alcohols and 2-alkylfurans when cysteine and ribose were heated with PC is more difficult to explain, but is probably due to Maillard reactants affecting the balance between the different pathways of lipid oxidation.

Although the Maillard reactants caused a reduction in one of the major classes of compounds (aldehydes) formed during lipid oxidation, this does not appear to constitute an overall antioxidative effect as other compounds derived from the oxidation of lipid showed little alteration. However, as aldehydes frequently possess very low odour thresholds, and are thought to be responsible for rancid odours in foods, their reduction by the Maillard reaction is likely to be important for food quality by whatever mechanism it occurs.

A comparison of the effects of the two purified classes of phospholipid, egg PC and PE, on the odour of the cysteine - ribose Maillard reaction showed that, despite the greater impact of PC upon the long-chain heterocyclics and alkanethiols, PE was more effective at generating a desirable meaty note in these model systems. Not only were there clear differences between the aromas of the lipid-containing Maillard systems, but the aromas of the four lipids heated alone also showed some interesting variations. Of particular note was the distinct "cooked chicken" aroma of the heated phospholipids, which was particularly pronounced and pleasant in the case of PE. Highly unsaturated aldehydes arising from the thermal oxidation of arachidonic acid are thought to contribute to the aroma of cooked chicken and may also be responsible for the aroma of heated PE.

Thus, of the lipids examined, PE conferred the most pleasant meaty aroma, whether heated alone or in the presence of cysteine and

ribose; the inclusion of these Maillard reactants changed the quality of the aroma from "cooked chicken" to an odour reminiscent of a roasted red meat such as lamb.

The results described in this Chapter suggest that the differences between the manner in which the various lipids participate in the Maillard reaction can be attributed partly to the nature of the phospholipid polar moiety and partly to dissimilarities in polyunsaturated fatty acid content. The amino groups of ethanolamine appear to modify the volatile products of lipid oxidation even in the absence of Maillard reactants, while the cysteine-derived amines and thiols may react with the more unsaturated products of oxidation to give long-chain heterocyclic compounds unique to this interaction. While the elucidation of the precise mechanisms of these reactions await further study, it is clear that the interaction between the pathways of lipid oxidation and the Maillard reaction will have a considerable impact on the formation of compounds responsible for the aroma of foods.

CHAPTER 4

SIGNIFICANCE AND FUTURE DIRECTION OF WORK

4. SIGNIFICANCE AND FUTURE DIRECTION OF WORK

4.1 SIGNIFICANCE OF RESULTS

All foods, whether animal or vegetable in origin, consist of a complex mixture of components. Lipids include not only triglycerides and phospholipids but also free fatty acids, di- and monoglycerides, lysophosphatides and steroidal compounds. As well as free amino acids, there are peptides and proteins serving a variety of functions: enzymes; structural proteins (actin, myosin and collagen); porphyrin-containing compounds (haemoglobin, myoglobin and chlorophyll). Carbohydrates include reducing and non-reducing sugars, disaccharides and oligosaccharides, glycogen (in animals) and starch (in plant material). In addition, all living organisms contain nucleotides, DNA, RNA, ionic salts and metal ions, vitamins and water (Lawrie 1985; Pomeranz 1987). Consequently, the chemical reactions responsible for the formation of aroma compounds in foods can be extremely complex. Many thousands of compounds have been identified amongst the aroma volatiles of foodstuffs, representative of a wide range of chemical classes and the origin of many of these remains uncertain.

Two reactions are responsible for the generation of a large proportion of the volatile aroma compounds in heated foods. The Maillard reaction between amino acids and reducing sugars gives many heterocyclic compounds containing oxygen, sulphur or nitrogen, while the thermal oxidation of lipids yields mainly aliphatic compounds. The mechanisms of these reactions have been the subject of much valuable and detailed work, reviewed in Sections 1.1 and 1.2, respectively. Many of the products of these reactions possess extremely low odour thresholds and make important contributions to the odours of foods.

Amino acids, sugars and lipids, the precursors of these reactions, are major constituents of most foods and often exist in close contact with each other; such circumstances must favour the occurrence of interactions between the Maillard reaction and lipid oxidation pathways. Such interactions have been the subject of a number of studies (Sec. 1.3), but relatively few have investigated

the role of such interactions in the generation of the desirable flavours of foods. Arnoldi *et al* (1987) reported that the formation of certain compounds (thought to be important indicators of cocoa quality) from leucine and valine is favoured by the presence of cocoa butter, while the presence of some lipid in meat has been shown to be a prerequisite of the desirable "meaty" aroma of the cooked material (Mottram and Edwards 1983; MacLeod and Ames 1987). These workers also noted that the quantities of some heterocyclic Maillard products were altered in defatted meat. Mottram and Edwards (1983) found that while the extraction of triglyceride from meat had little effect on aroma, the additional removal of phospholipids altered the aroma so that it was no longer meaty in character. However, whether phospholipid itself was necessary for the formation of meat aroma or whether a small amount of any lipid would have the same effect required further elucidation.

In Chapters 2 and 3 it has been shown that the odours of the phospholipid-Maillard systems differed from those of the lipids or Maillard reactants heated alone. In particular, the addition of phospholipid, but not triglyceride, to the Maillard reaction between cysteine and ribose considerably enhanced the meatiness of the aroma. These results support the hypothesis (Mottram and Edwards 1983) that interactions between phospholipids and the Maillard reaction are important for the generation of the compounds responsible for meat flavour. Of the lipids studied, egg PE conferred the most pleasant meat-like aroma, and phosphatidylethanolamine may have specific importance for the formation of the desirable aroma of cooked meat.

The inclusion of a phospholipid also has a marked influence on the aroma of the individual odours eluting from a gas chromatograph; phospholipid increases both the number and range of odours detected, adding "chicken-like" aromas to the glycine + ribose system and increasing the number of odours described as "meaty" in the cysteine-containing system (Chapter 2).

The formation of aroma volatiles from glycine or cysteine-containing Maillard reactions, and related systems, in the absence of lipid have been the subject of a number of reports (Mulders 1973; Shibamoto and Bernhard 1977, 1978; Hartman *et al* 1984b; Shu *et al* 1985a,b; Wong and Bernhard 1988). Nevertheless, many

compounds have been detected in the studies described in Chapter 2 which have not been reported previously in such model systems. These include a series of alkylfurfurals from the reaction between glycine and ribose and a number of acylthiophenes, dihydro-3(2H)-thiophenones, 1,2-dithiolan-4-ones, 1,2-dithian-4-ones and thienothiophenes from the cysteine + ribose Maillard system.

In both reactions it was shown that the addition of phospholipid increases the number of compounds detected and adds certain substances specific to the interaction between these two reactions. These include 2-pentylpyridine, 2-alkylthiophenes, alkenylthiophenes, pentylthiapyran and 1-alkanethiols.

Ho and co-workers (Carlin *et al* 1986; Ho *et al* 1987) have identified a number of classes of long-chain heterocyclic compounds in the volatiles from deep fat-fried foods, requiring the presence of both lipid and Maillard reactants for their formation. It was presumed that these compounds arose from the interaction of the oil used for frying with components of the food. However, these studies do not differentiate between products derived from the frying oil and those derived from triglycerides or phospholipids present in the foods themselves.

A number of studies have used lipid-containing model systems to investigate the formation of such long-chain heterocyclic compounds (Brietbart and Nawar 1981; Henderson and Nawar 1981; Chiu *et al* 1990). Apart from recent investigations on the role of corn oil in the formation of volatiles from extruded corn (Huang *et al* 1987; Bruechert *et al* 1988), these studies have concentrated on triglycerides containing mainly (or totally) saturated fatty acids. In contrast, the role of the highly unsaturated phospholipids in the generation of such aroma volatiles has received little attention. As major structural components of cell walls, phospholipids are frequently in close proximity to the aqueous cell contents; thus, these lipids may be expected to be particularly prone to interactive reactions with sugars and amino acids.

Preliminary studies (Whitfield *et al* 1988) indicated that the inclusion of PC in aqueous Maillard reactions modified the amounts of various products formed and resulted in the formation of a number of long-chain heterocyclic compounds. These studies have

now been extended to compare the effects of including each of three phospholipids and a triglyceride in the Maillard reaction between cysteine and ribose.

The work described in Chapter 3 examines the effect on both the Maillard and lipid oxidation reactions of the presence of reactants, intermediates and products of the other, and provides new insight into the extent and consequences of such interactions. Distinct differences were apparent between the effects of the different lipids. The three phospholipids studied had a greater effect on both Maillard reaction products and aroma than did triglyceride. Differences were also observed between the phospholipids; while egg phosphatidylcholine gave the greatest yield of long-chain heterocyclic compounds, egg PE and beef phospholipid gave the greatest suppression of heterocyclic thiols as well as the most intense meaty aromas. It is suggested that both the ethanolamine group and polyunsaturated fatty acids can contribute to the differences in the way that individual phospholipids participate in the Maillard reaction.

The results described in this Thesis demonstrate that, when lipid and Maillard reactants are heated in close contact, the interaction between them is likely to modify the balance of odour compounds produced. Such situations are not confined to meat, since all biological materials contain phospholipid; interactions could occur during the cooking of many foods, including baked products (pastry, biscuits, cakes) and cooked vegetables and fruit. The effects of these interactions are likely to be greatest where the lipid has a high proportion of reactive polyunsaturated fatty acids. Not only is the balance between the volatile products of these reactions likely to be altered, but extra compounds, formed uniquely from the interaction between these reactions, may be formed. Where the volatile compounds altered by lipid-Maillard interactions have very low odour thresholds and are key odour components, the final odour characteristics of the food will depend upon these effects.

4.2 FUTURE DIRECTION OF WORK

The studies described in this Thesis have raised a number of issues which would benefit from further investigation. The formation of some of the long-chain heterocyclic compounds is postulated to require the presence of polyunsaturated fatty acids or their aldehydic breakdown products. Thus, the increased interaction of the phospholipids can be accounted for, in part, by their higher content of such fatty acids. However, the phospholipids with the highest levels of polyunsaturated fatty acids do not give the most long-chain heterocyclic compounds, although they do cause the greatest depletion of heterocyclic thiols. Apparently, the nature of the phospholipid polar group (ethanolamine or choline) also has an effect; this appears to be primarily through the reaction of the ethanolamine amino group with aldehydes.

The precise role of polyunsaturated fatty acids and phospholipid polar groups in the interactive reactions occurring between the Maillard reaction and lipid oxidation could be further investigated by the use of synthetic phospholipids containing either the same fatty acid composition and different polar groups, or the same polar groups and different fatty acids. However, such lipids are extremely expensive and similar information might be gained by comparing model reactions containing Maillard reactants, individual fatty acids and either ethanolamine or choline. Such investigations would clarify further the role of individual phospholipid components in the interactions occurring between the Maillard reaction and phospholipids.

The most distinct "meaty" aromas were generated by heating egg PE, alone to give an aroma of cooked chicken, and with cysteine and ribose to give an aroma more reminiscent of a cooked red meat. A study of these aromas using dilution techniques may indicate the compounds primarily responsible for these odours. Comparison with the LRI values of the key odour compounds in cooked chicken and beef evaluated in the same way should indicate whether the same compounds are responsible in both meat and model systems. If so, their subsequent study may be facilitated by the use of such model systems.

Certain classes of compounds discussed in the previous Chapters are of particular interest as they contain compounds not reported previously in foods or model systems. The novel series of alkylfurfurals, detected as major products of the Maillard reaction between glycine and ribose were tentatively identified as 2- rather than 3-furfurals. However, the possibility of 3-substitution cannot be precluded; in order to establish unequivocally the position of the formyl group it will be necessary to collect sufficient of these compounds for NMR studies.

Five of the six furfurals detected contain more than five carbon atoms, but were still formed from ribose heated alone. Preliminary studies suggest that the amount of 2-furfural is reduced on addition of glycine, ethylmethylfurfural is unchanged and the dimethyl- and trimethylfurfurals are increased. The pathways responsible for the formation of these compounds may be related to those leading to acylthiophenes and dihydro-3(2H)-thiophenones and other compounds with more than five carbon atoms detected in the reaction between cysteine and ribose. The possibility that the formation of these compounds involves aldol condensation reactions between sugar fragments, and possibly amino acid breakdown products, requires further elucidation. The precise structures and origin of some of the pyrroles detected in the Maillard reaction between glycine and ribose also warrant further study.

The 3-furanthiols, their thiophene analogues, disulphides and related compounds are of special interest due to their low odour thresholds and their perceived importance for meat flavour. The meaty character of these compounds has been exploited in synthetic meat flavourings for some years (Evers 1971; van den Ouweland and Peer 1972; Evers *et al* 1976); however, their presence in meat itself has only recently been established, due to their extremely low concentrations (MacLeod and Ames 1986; Gasser and Grosh 1988; Farmer and Patterson 1990).

Two aspects of the behaviour of these compounds in the reactions described in the previous Chapters seemed curious and therefore worthy of further study. Although several furyl and thienyl disulphides were detected among the headspace volatiles from the cysteine + ribose + phospholipid reaction studied in Sec. 2.3, these compounds were not detected in the four similar systems

described in Sec. 3.2. One of the differences between these systems was the strength of the phosphate buffer; while a 0.2M buffer was used for the earlier reactions, this was modified to a 0.5M buffer for the later reactions to minimize the drop in pH during reaction. Investigation of reaction mixtures using a 0.1M buffer, which gave a greater pH drop during heating, showed higher levels of these disulphides than systems containing greater buffer concentrations. It appeared that the formation of these compounds is pH dependent and that the slightly higher final pH using the 0.5M buffer (Section 3.2) may have precluded their formation. Subsequent studies have examined the formation of four disulphides at five pH values over the range pH 4.5 to 6.5 (Farmer and Mottram 1990b). The results of this investigation showed that these compounds are indeed extremely pH dependent, with maximum formation at pH 4.5 reducing to zero at pH 6.0 and above. The flavour intensity of meat is known to increase with decreasing pH; for example, 'dark-cutting' beef with a high pH has poor flavour (Lawrie 1985). The marked pH dependency of the generation of furyl and thienyl disulphides may offer an explanation for the relationship between pH and flavour formation in meat.

A second feature of the 3-furanthiols and their sulphides and disulphides was that, although they are thought to be important in meat flavour, and are known to have strong meaty odours with extremely low odour thresholds, the increased "meaty" aroma detected in the presence of phospholipid was concomitant with a reduction of these compounds in the headspace. One explanation for this observation is that, at high concentrations, the odours of these compounds are strongly sulphurous and that their odours are more pleasant when reduced by the presence of phospholipid. An alternative hypothesis is that other related compounds, also with low odour thresholds, are formed only in the presence of phospholipid. A search has been made for lipid-dependent compounds containing the 2-methyl-3-furylthio moiety. The tentative identification of two such compounds, 1-(2-methyl-3-furylthio)propan-2-one and 2-methyl-3-furyl tetrahydrofuryl sulphide, has been reported recently (Farmer and Mottram 1990b); these and other related compounds should be an important area for future work.

CHAPTER 5

CONCLUSIONS

5. CONCLUSIONS

More than 200 volatile compounds have been identified in Maillard reactions between glycine or cysteine, ribose and phospholipid. These include a number of compounds not reported previously in model systems or in foods. In the absence of phospholipid, heterocyclic compounds predominate; a series of 2-acylfurans dominate the headspace of the reaction between glycine and ribose, while major volatiles from the cysteine-containing Maillard reaction include furanthiols, thiophenethiols, dihydro-3(2H)-thiophenones and acylthiophenes. The inclusion of phospholipid considerably increases the number of volatile components, adding not only the largely aliphatic products of lipid oxidation, but also compounds specific to the interaction of the lipid in the Maillard reaction, such as 2-pentylpyridine, 2-alkyl- and alkenylthiophenes and 1-alkanethiols.

The addition of phospholipid also increases the number and range of individual odours detected in the reaction mixtures and thus had a marked influence on the overall aroma of both Maillard systems. The inclusion of phospholipid in the cysteine + ribose reaction mixture adds a persistent meat-like note to the overall odour, which is reflected in the increased number of individual "meaty" aromas detected in the GC effluent.

A comparison of the effect of four different lipids on the Maillard reaction between cysteine and ribose demonstrates marked dissimilarities between the behaviour of the four lipids. The most noticeable distinction is between the effect of triglyceride and the three phospholipids; most of those compounds whose mechanisms of formation are susceptible to lipid intervention are more affected by the presence of a phospholipid than the beef triglyceride. Furthermore, unlike the triglyceride, the three phospholipids confer a distinctive meaty aroma on this reaction mixture. These results corroborate the findings of previous work, which showed that the removal of phospholipid from muscle reduced the meaty aroma of cooked beef and pork.

In addition to the clear difference between the triglyceride and the phospholipids, there are also variations in the way that the three phospholipids interact in the Maillard reaction. A

comparison of the two purified classes of phospholipid represented in this study, egg phosphatidylcholine and phosphatidylethanolamine, shows that, despite the greater impact of phosphatidylcholine upon the long-chain heterocyclic compounds and alkanethiols, phosphatidylethanolamine is more effective at generating a desirable meaty note in these model systems.

Not only does the addition of certain lipids modify the volatile products of the Maillard reaction, but the presence of Maillard reactants also alters the amounts of compounds derived from lipid degradation; the generation of aldehydes, especially unsaturated aldehydes, is much reduced by the presence of cysteine and ribose. However, other oxidation products are little changed and an overall antioxidative effect is not observed. The formation of aldehydes is also reduced in those phospholipids containing ethanolamine groups. The difference in behaviour between the various lipids appears to be due to dissimilarities in their fatty acid compositions and phospholipid polar moieties.

Both Maillard and lipid oxidation reactions are reponsible for the formation of compounds with extremely low odour thresholds which contribute characteristic odours to many foods. The extensive changes to the products of both these reactions when phospholipids and Maillard reactants are heated together suggests that phospholipid-Maillard interactions may be of importance for flavour formation, not only in meat, but in many foods.

CHAPTER 6

EXPERIMENTAL

6. EXPERIMENTAL

6.1 MATERIALS

Glycine, L-cysteine and D(-)ribose were purchased from Sigma Chemical Company (Poole) as were the three egg phospholipids required. A phospholipid fraction (L-α-phosphatidylcholine, type IX-E, from fresh frozen egg yolk), containing up to 30% phosphatidylethanolamine as well as the phosphatidylcholine, was used in the glycine or cysteine + ribose systems, described in Chapter 2. L-α-phosphatidylcholine (Type III-E from egg yolk, approx. 99%) and L-α-phosphatidylethanolamine (Type III from egg yolk, approx. 98%) were required for the study of the interaction of different lipid classes in the cysteine + ribose reaction (Chapter 3). The beef triglyceride and beef phospholipid also utilised for this experiment were extracted from beef adipose tissue and muscle respectively (see Sec. 6.1.2).

Reference chemicals were either purchased from reliable sources or acquired as gifts from flavour laboratories; the sources of these compounds are indicated in Appendix I. Other reagents and solvents were purchased from the normal laboratory chemical suppliers and were of Analar standard whenever possible.

6.1.1 Buffers

Phosphate buffers were prepared from $Na_2HPO_4.12H_2O$ and $NaH_2PO_4.2H_2O$ (BDH Chemicals Ltd, Poole) in glass distilled water. A 0.2M, pH 5.7 buffer was used for the experiments in Chapter 2, while a 0.5M, pH 5.6 buffer was used for the comparison of lipids in Chapter 3. This modification was made to minimise the change of pH during reaction. The pH of the cysteine + ribose mixture decreased by 0.9 pH unit when heated in 0.2M buffer and by 0.4 pH unit in 0.5M buffer.

6.1.2 Extraction of beef lipids

Subcutaneous fat (from the top of the rump) and *M. semimembranosus* were obtained from a Hereford x Friesian steer, slaughtered at approx. 15 months. The lean muscle was homogenized in a domestic food processor and vacuum packed in 100g portions. Both tissues were stored at -20°C until required.

Beef triglyceride

Beef adipose tissue (100g) was homogenized to a paste in a Moulinette blender, melted over low heat and strained through two layers of muslin. The rendered fat was dissolved in chloroform : methanol (2:1) to give a final volume of 300 ml, shaken with distilled water (60ml) and left overnight before discarding the aqueous phase. The organic layer was dried with Na_2SO_4, filtered and the solvent removed by rotary evaporation. The lipid was dissolved in warm hexane (100 ml, 40°C) and Florisil (8g) was added to remove phospholipid, mono- and diglycerides as well as free fatty acids. The mixture was mixed by swirling and chloroform (100 ml) added immediately. After 5 min, with occasional stirring, the solution was filtered and the solvent removed. The triglyceride was stored under nitrogen at -20°C until required.

Beef phospholipid

Total lipid was extracted from *M. semimembranosus* using a method based on the procedure of Folch *et al* (1957) and adaptations by Piotrowski *et al* (1970) and Hornstein *et al* (1961).

Muscle tissue (100g) was homogenized with methanol (167 ml) in a homogenizer (Ystral, Ballrechten-Dottingen, FRG) for 4 min. Chloroform (333 ml) was added, followed by further homogenization for 4 min. The slurry was filtered through two washed filter papers (Whatman No 41), to facilitate the removal of the residue. This was re-extracted with 500 ml chloroform : methanol (2:1) for 4 min and filtered as before. The combined extracts were washed with aqueous sodium chloride (0.58%, 200 ml) and left to stand overnight in the dark. The aqueous phase was removed, and discarded, using a Pasteur pipette connected to a water pump via a

conical flask trap. Methanol was added to dissolve the "fluff" at the interface and the solution was dried with anhyd. Na_2SO_4, filtered and the solvent removed by rotary evaporation. The lipid was dissolved in 20 ml chloroform : methanol (20:1).

The phospholipids were separated from triglyceride on a silicic acid column by a method adapted from Hornstein *et al* (1961). Silicic acid, which had been heated overnight at 130^oC, was slurried into a glass column (2 cm ID) fitted with a sintered disc. When the column had settled, anhyd. Na_2SO_4 was added to a depth of 1cm. The lipid sample was placed on the column and eluted with chloroform : methanol (20:1) to remove mono-, di- and triglycerides and fatty acids. The column was then eluted successively with 150 ml chloroform : methanol (1:1) and 300 ml methanol. These last two fractions contained phospholipids and were combined, taken to dryness on a rotary evaporator and dissolved in chloroform : methanol (2:1). Insoluble material appearing at this stage was presumed to be proteinaceous material derived from a class of lipoproteins, previously found to be unstable under these conditions (Hornstein *et al* 1961). The phospholipid fractions from four extractions (100g muscle each) were combined and repeatedly taken to dryness, redissolved in chloroform : methanol (1:1) and centrifuged at 1700g for 5 min, until a constant weight of lipid was obtained. The final sample was stored in chloroform at -20^oC under nitrogen.

6.1.3 Analysis of lipids

Samples of purified beef triglyceride and beef phospholipid were tested for contamination by each other or by free fatty acids, mono- or diglycerides using thin layer chromatography on 0.25 mm, SIL-G plates (Macherey-Nagel & Co, Duren, West Germany), eluted with hexane : diethylether : acetic acid : methanol (100 : 20 : 2 : 1.8). In order to estimate the level of any impurities each fraction was applied to the tlc plate in four different amounts (100 μg, 10 μg, 3 μg, and 1 μg). The plates were developed using iodine and 2',7'-dichlorofluorescein.

The final samples of beef triglyceride and beef phospholipid were analysed for organic nitrogen by the Kjeldahl method (Bradstreet

1965), suitably modified for micro quantities using a Markham micro-Kjeldahl still (Gallenkamp, Loughborough).[a]

The fatty acid composition of each of the four lipids studied in Chapter 3 was determined, after saponification and methylation using diazomethane[a], by gas chromatography on fused silica capillary columns, coated with either CPWAX57CB (26m x 0.32mm ID) or CPWAX52CB (50m x 0.32mm ID), purchased from Chrompak UK Ltd, London. Identification of fatty acid methyl esters was achieved by comparison with standards and by GC-MS analyses using both electron impact and chemical ionization. The mass spectrometric conditions for electron impact ionization were similar to those used for the rest of the work reported herein. Chemical ionization-MS was performed using methane as the reagent gas; this technique gave a clear $M+1^+$ ion for the methyl esters and thus enabled the number of carbon atoms and double bonds in each fatty acid to be established. The position of the double bonds was determined by co-injection with the authentic material or by comparison with literature mass spectra.

The fatty acid composition of the phospholipid fraction used in Chapter 2 was also analysed and the nitrogen content determined by a micro-Kjeldahl method.

6.2 PREPARATION OF REACTION MIXTURES

6.2.1 Reaction of glycine or cysteine with ribose and phospholipid (Chapter 2)

Glycine or cysteine (100mg) and ribose (90mg) were dissolved in 20ml phosphate buffer, with and without phospholipid (300mg) and were placed in 100ml thick-walled 'Duran reagent bottles' fitted with PTFE-lined 'Resinol' screw caps (BDH Ltd, Dagenham). The reaction mixtures were treated at 40°C for 1h in an ultrasonic bath to melt and disperse the lipid, prior to heating in an autoclave (Certoclav, Strands Scientific, Nottingham) at 140°C (270 kPa) for 1 h. A solution of ribose only in buffer was treated in the same way. The headspace volatiles were collected from the

a I acknowledge gratefully the assistance of Mr A. Crosland and Mr R. Davison for performing the micro-Kjeldahl analyses and of Dr M. Enser and Mrs J. Roberts for the saponification and methylation of the fatty acids.

whole sample (20ml) as well as from diluted mixtures containing 2ml reaction mixture and 18ml phosphate buffer, to obtain clear mass spectra and odour assessments for very abundant components.

6.2.2 Interaction of four lipids in reaction between cysteine and ribose (Chapter 3)

Table 6.2a: Experimental plan

	With cysteine + ribose	Without cysteine + ribose (lipid alone)
Without lipid	Reaction mixture no.1	2 (buffer only)
With beef triglyceride	3	4
With beef phospholipid	5	6
With egg PC	7	8
With egg PE	9	10

Cysteine and ribose were heated both with and without each of the four lipids in phosphate buffer at pH 5.6; each lipid was also heated alone under the same conditions, as indicated in Table 6.2a. Reaction mixtures were prepared in quadruplicate.

For the lipid-containing reaction mixtures (3-10), sufficient of the lipid solutions to give 30 mg lipid were placed in previously flamed 2.5 ml freeze-drying ampoules made from Pyrex glass (Severn Science Ltd, Bristol), and the solvent removed under a stream of N_2 with gentle warming (40-50°C). Cysteine (5 mg ml^{-1}) and ribose (4.5 mg ml^{-1}) were dissolved in phosphate buffer (0.5M, pH 5.6) and 2 ml of this solution was added to each of the odd-numbered ampoules (Table 6.2a); buffer (2 ml) was added to each even numbered ampoule. Nitrogen was blown over each of the samples for 2 min and the ampoules flame-sealed. The ampoules were subjected to ultrasonication (40°C, 1 hr) to aid the dissolution of the lipids.

Reaction mixtures were heated for 1 hr at 140°C (270 kPa) in an autoclave (Certoclav). The mixtures were then stored at -20°C for 1-2 days if not required for immediate headspace collection. Each reaction mixture (2 ml) was diluted to 20 ml in phosphate buffer for the isolation of volatiles.

6.2.3 Choice of reaction conditions

The model systems used in these studies were designed to approximate the composition of meat. The concentrations of carbohydrate, amino acids, phospholipid and water in red meat have been listed as 0.3%, 0.35%, 1.0% and 75%, respectively (Lawrie 1985). These values were converted into mass of each component in 20 ml aqueous reaction mixture to give experimental values of 90 mg sugar, 100 mg amino acid and 300 mg phospholipid. In meat, one individual amino acid will not make up the total free amino acids and all the carbohydrate will not be ribose. However, initial trials demonstrated that these concentrations gave interesting aromas and, therefore, were used henceforth.

Comparison of the odours and volatile compounds at reaction temperatures of 100°C, 120°C and 140°C showed that both were considerably depleted at the lower temperatures; thus, a temperature of 140°C was used throughout these studies.

6.3 ISOLATION OF VOLATILES

The volatile compounds produced by the heated reaction mixtures were collected in glass-lined stainless steel traps containing 2.6mg Tenax-GC (Scientific Glass Engineering Ltd, Milton Keynes) using a headspace concentration technique. The traps were conditioned at 250°C for 40 min, prior to use. In the experiments described in Chapter 3, an internal standard containing alkanes (C_{10} - C_{24}, 10 ng each) and 1,2-dichlorobenzene (65 ng) was placed on the trap in 1 μl ethanol and the solvent removed by the passage of N_2 (50 ml min^{-1}) for 5 min. After heating in the autoclave, each reaction mixture was cooled and placed in a 250 ml Erlenmeyer flask, with buffer as required to give a final volume of 20 ml.

The Erlenmeyer flasks were fitted with 30 mm screw joints to take a sliding joint with PTFE seal (SVL fittings; J. Bibby Science Products, Stone) and Dreschel head as illustrated in Figure 6.3A. The temperature of the flask was maintained at 60°C, the contents stirred and the volatile compounds swept onto the Tenax trap with a stream of N_2 (50 ml min^{-1}) for 30 min. After collection, residual water was removed from the traps with N_2 (50 ml min^{-1}, 5 min). The nitrogen supply ('oxygen free'; BOC Ltd, Bristol) was passed through granular charcoal (10-18 mesh; BDH Chemicals Ltd, Poole) to remove traces of organic compounds before use.

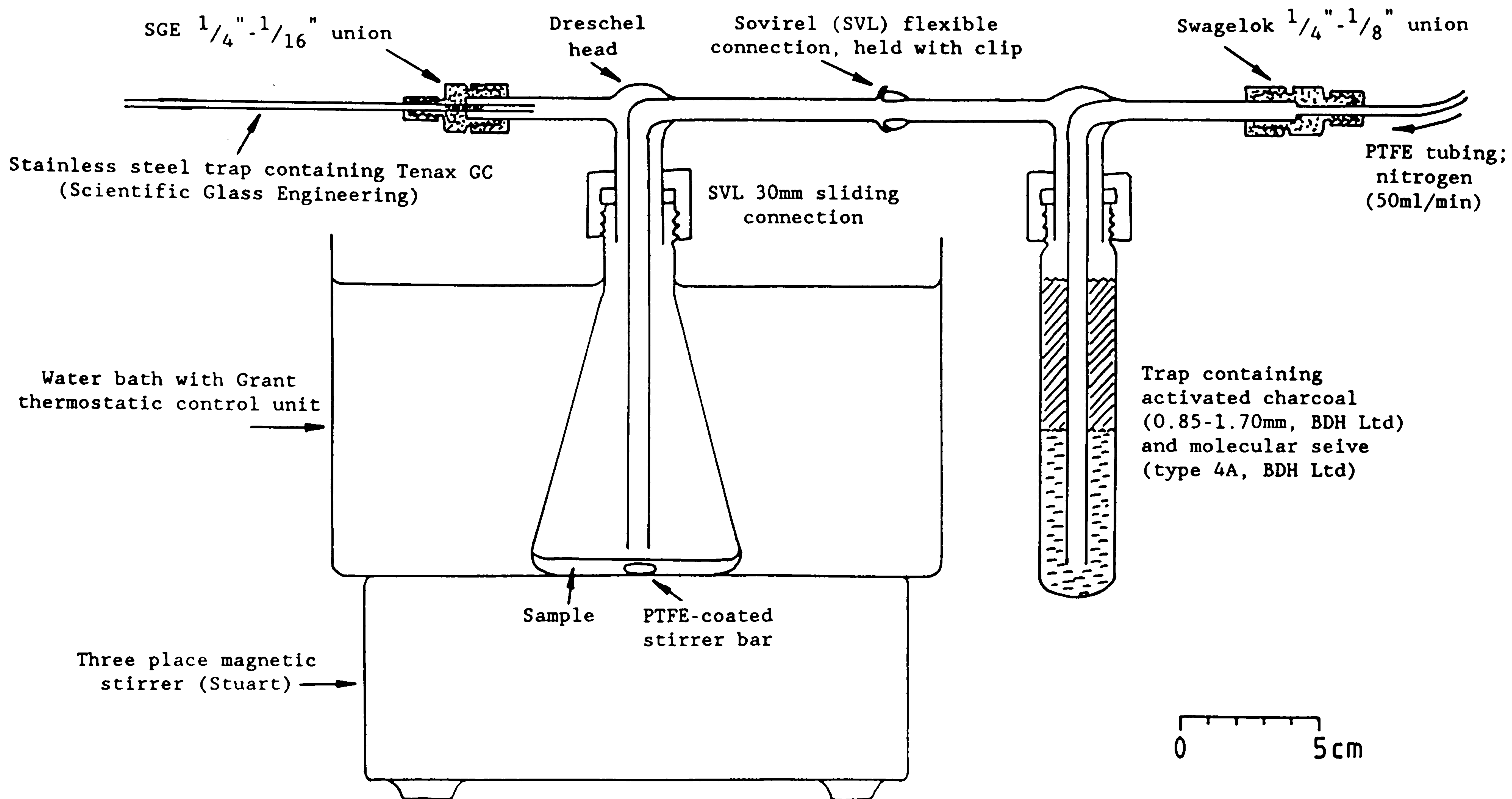
Authentic reference compounds were analysed under the same conditions as the collected samples by adding a known amount of a compound (*ca* 40ng in 1 μl hexane) to a Tenax-GC trap, to which alkanes and the internal standard (1,2-dichlorobenzene, 65 ng) had been applied. Residual solvent was removed with a stream of N_2, as described above.

6.3.1 Choice of technique

The headspace concentration technique described above is one of a number of techniques available for the isolation of volatile aroma compounds. These have been extensively reviewed (Charalambous 1978; Cronin 1982; Peyron 1982; Maarse and Belz 1985) and will be discussed only briefly here. Such methods include direct injection of headspace volatiles, cryogenic techniques, solvent extraction, simultaneous distillation/extraction, and trapping on to an adsorbent.

Direct Injection, also known as 'static headspace analysis', involves the direct sampling of headspace volatiles with a syringe and their injection on to a GC column. Such a technique is not entirely suited to use with capillary GC as the slow flow rates can cause excessive peak broadening with conventional injection systems; however, this problem can be overcome by cooling the front of the column, or by using on-column injection techniques to focus t sample. While this method has the benefit of presenting the sample to the GC in exactly the composition experienced by the nose, the low quantities of injected volatiles means that its use is limited by the low sensitivity achieved (Leahy and Reineccius 1984; Gordon 1987). For this reason other techniques

Figure 6.3A: Diagram of apparatus used for the collection of volatiles from aqueous reaction mixtures on to traps containing Tenax GC.



have been developed which involve concentration of the aroma volatiles.

Cryogenic techniques have been used for the extraction and concentration of volatiles using a wide range of apparatus; in a typical system, a vacuum is used to vaporize the volatile components of the sample, which are then condensed on a cold finger, usually cooled with liquid nitrogen. Such techniques are inherently gentle and minimise thermal breakdown or further reaction of the volatiles collected. However, most foods or model systems also yield large amounts of water, which is also condensed; therefore it is usually necessary to extract with solvent prior to analysis by GC.

Solvent Extraction techniques include not only the traditional batch methods but also continuous extraction methods. Leahy and Reineccius (1984) have compared the efficacy of solvent extraction techniques with other methods for the extraction of a mixture of volatile organic compounds. They concluded that solvent extraction techniques gave the best overall recovery over the range of volatilities and compound classes studied. However, these techniques are limited to systems which do not contain lipids.

Simultaneous distillation/extraction eliminates the problem of co-extraction of lipids by distilling both sample volatiles and solvent prior to extraction, and takes place between the condensed phases. The most commonly used version is the 'Likens-Nickerson' apparatus. A disadvantage of this technique is that heat is required for the steam distillation of the volatiles and this increases the risk of their thermal degradation. The application of a vacuum is said to minimise heat-induced artifact formation (Leahy and Reineccius 1984). A disadvantage of solvent extraction methods is that the aroma volatiles collected are not in the same proportions as experienced by the human nose as the headspace above the food or model system is not directly sampled.

Headspace concentration techniques have been developed to enable sampling of the headspace gases while overcoming the problems of insensitivity of direct injection techniques. Although the sample obtained is not entirely representative of that detected by olfaction, due to the continual disturbance of the equilibrium, it is better than those obtained by solvent extraction. These

techniques involve purging of the headspace with an inert gas, followed by trapping of the volatiles, usually on to a suitable adsorbent. This may be a porous polymer, such as Tenax, Chromosorb, Porapak or Amberlite XAD, or, in some cases, activated charcoal. The behaviour of a number of adsorbents has been compared by Schaefer (1981). Tenax-GC was found to produce cleaner blank than most adsorbents, while recent studies have suggested that Tenax-TA may give even fewer contaminants (MacLeod and Ames 1986c). Volatile compounds may also be transferred on to such traps by the application of a vacuum, as used in the studies of Galt and MacLeod (1984) and Vercellotti *et al* (1988). Porous polymeric materials have the advantage that they have little affinity for water, enabling the selective concentration of organic components from an aqueous medium. However, they can be inefficient absorbers of very low boiling components. In the comparative studies of Leahy and Reineccius (1984), trapping on to Tenax was found to give the greatest sensitivity, permitting detection of compounds at concentrations of 1 part in 10^{12}.

A relatively recent development in the field of aroma isolation is that of **supercritical fluid extraction**, which involves the extraction of organic compounds using a supercritical fluid, usually CO_2. Such fluids have strong solvating abilities, low viscosities and high diffusibilities, aiding rapid partition. The low temperatures of use minimise degradation of analytes while the gaseous nature of the medium at room temperature facilitates solvent removal (Hawthorne 1988). This and other recent developments in the isolation of volatile compounds from foods have been reviewed by Risch and Reineccius (1989).

The technique of choice for a given application depends upon the medium from which the volatile compounds are to be extracted, the lability of the compounds of interest and the purpose of the subsequent analysis.

The technique chosen for the present studies had to concentrate the organic volatiles selectively from aqueous model systems without collecting water vapour. Sufficient sample was required for the identification of the volatile products by GC-MS and also the less sensitive techniques of high resolution GC-MS and GC-IR.

For these reasons, the headspace concentration technique described above was deemed best suited to the work.

Tenax-GC was chosen as the absorbent because of its high temperature stability (up to 380-400 ^{o}C), which makes it one of the best porous polymers for temperature desorption (MacLeod and Ames 1986c).

6.4 INSTRUMENTAL

6.4.1 Gas chromatography

Gas chromatography was performed using Carlo Erba gas chromatographs (models 4130, 4200) for GC-MS and GC-odour assessment and a Hewlett Packard instrument (5890) for GC-IR analyses. WCOT fused silica capillary columns (50m x 0.32mm i.d.; 0.19 μm film thickness) coated with CPWAX57CB (Chapter 2) or CPWAX52CB (Chapter 3), supplied by Chrompak UK, Ltd were used. All gas chromatographs, except that used for GC-MS, were fitted with flame ionization detectors.

Each GC was fitted with a 'Unijector' (Scientific Glass Engineering Ltd, Milton Keynes) in addition to the Grob 'split/splitless' injector. The Unijector is an inert glass-lined injector system which may be modified for use in several modes; in these studies it was used in the 'headspace/concentrator' mode, for the desorption of volatiles from glass-lined stainless steel traps containing Tenax-GC. The structure and mode of action of the Unijector are illustrated in Figures 6.4A and B.

During desorption, the trap, containing a collected sample, was placed in the heated Unijector (250°C) and the helium carrier gas (80 kPa, giving a flow over the column of approx. 1 ml min^{-1} and a purge flow of 15 ml min^{-1}) directed through the trap. The volatiles were carried on to the GC column where they were condensed by cooling a 10 cm length of the front of the column with solid CO_2. The 'high purity' helium used as a carrier gas was

Figure 6.4A: Diagram of 'Unijector' (Scientific Glass Engineering)

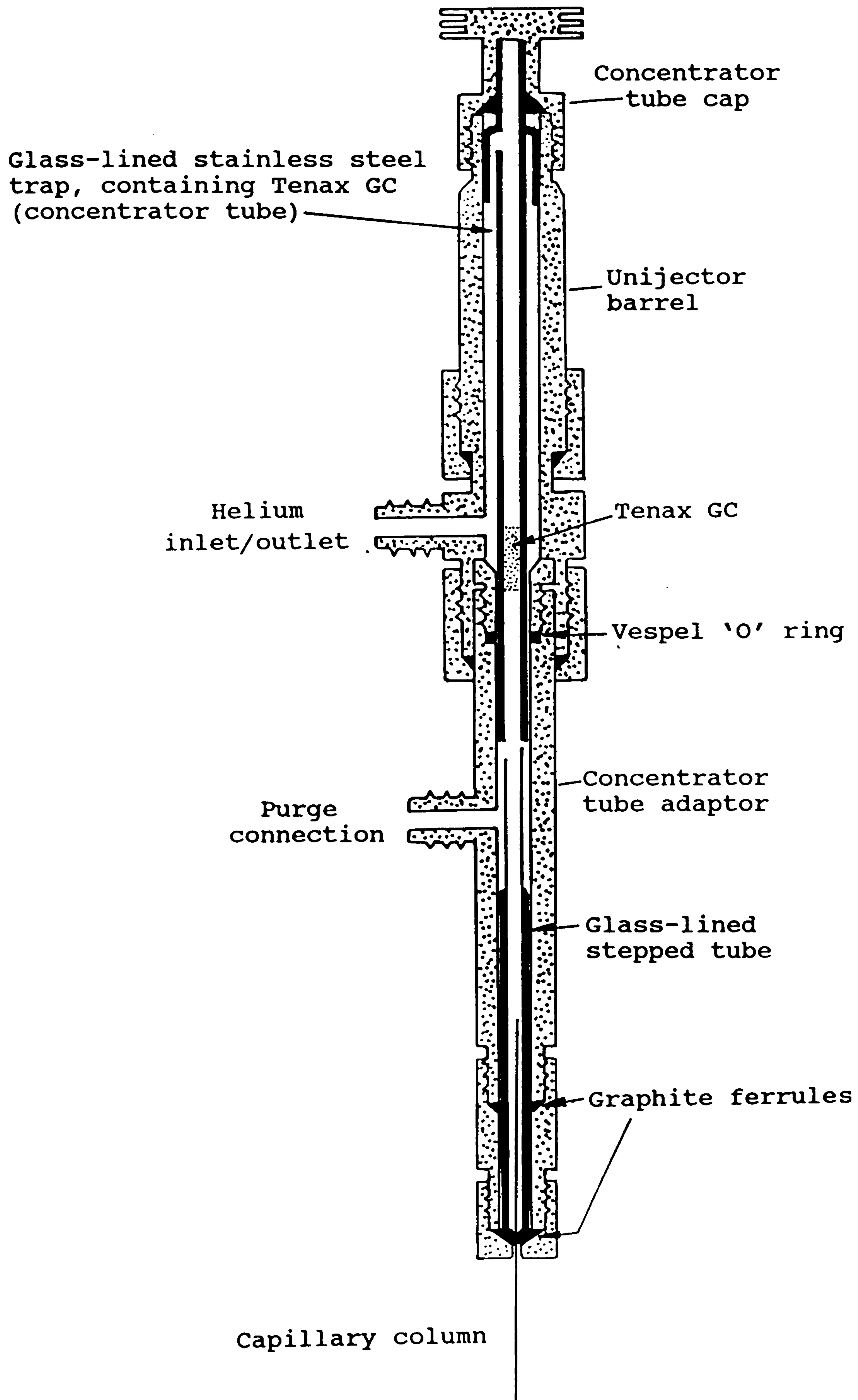
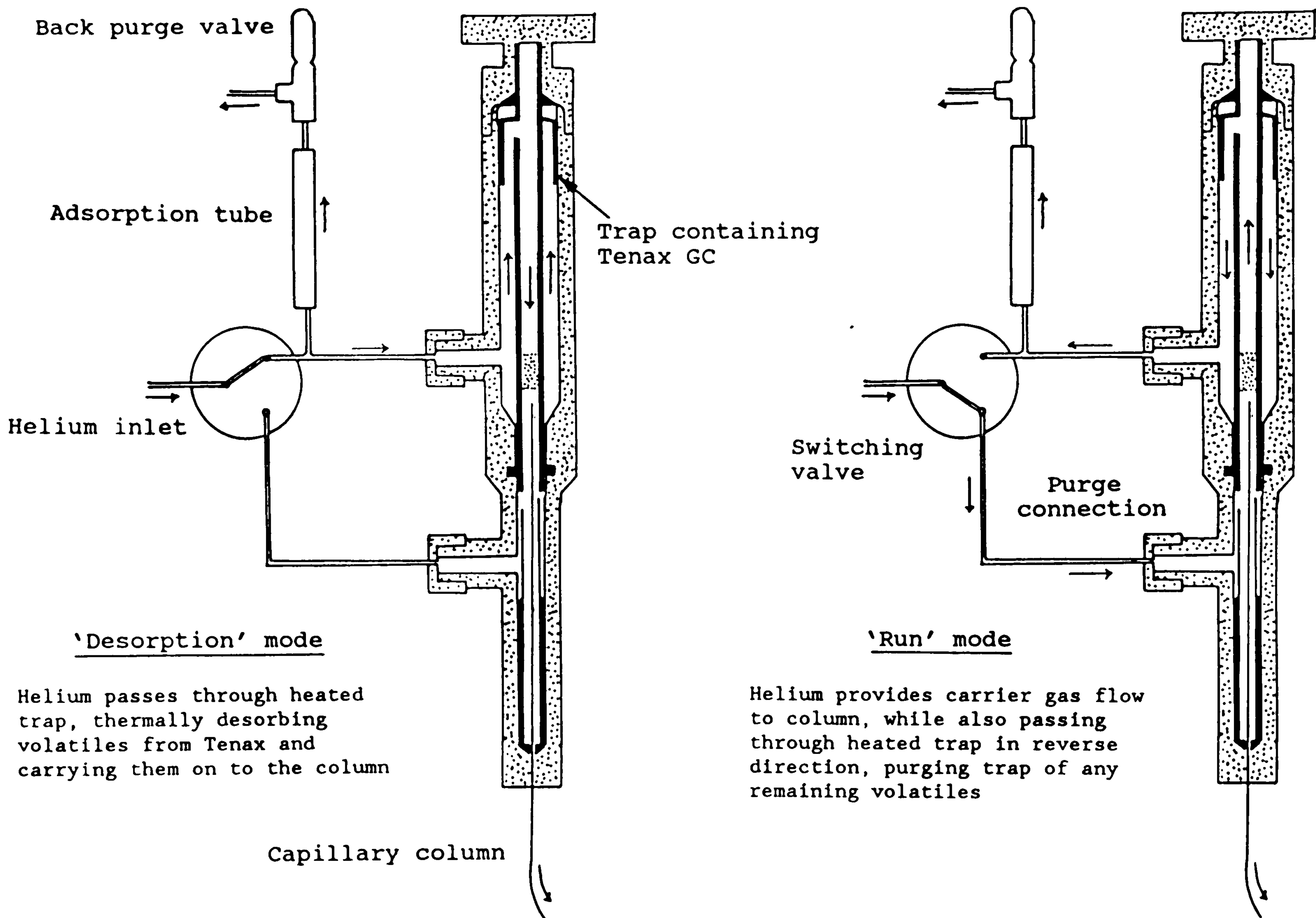


Figure 6.4B: Mode of action of 'Unijector' (Scientific Glass Engineering)



obtained from Gas and Equipment, London. For the studies described in Chapter 2, desorption was carried out for 15 min; subsequent investigation showed that a desorption time of 5 min was adequate and this shorter period was used for the experiment described in Chapter 3. After desorption, the oven was heated rapidly to $60^{o}C$ and maintained at this temperature for 5 min before increasing to $200^{o}C$ at $4^{o}C$ min^{-1}.

In the Carlo Erba instruments the Unijector was mounted within the injection port heater of the GC with the lower portion of the Unijector projecting into the GC oven. It was found that the lower part of the Unijector did not attain the requisite desorption temperature, which could result in poor desorption of higher molecular weight compounds. The problem was overcome by applying supplementary heating in the form of an electrical heating tape (Isopad Ltd, Borehamwood, Herts), wound round the lower regions of the Unijector, within the GC oven. The temperature of this extended heated zone was controlled by a thermocouple and proportional feedback controller. With this modification, it was possible to desorb up to the C_{28} n-alkane without difficulty.

6.4.2 Gas chromatography-mass spectrometry

Analyses were performed on a Carlo Erba 4130 gas chromatograph coupled to a Finnigan 4000 mass spectrometer equipped with an Incos 2100 data system. The volatile compounds trapped from each reaction mixture were desorbed on to the front of the column as described above. The column was connected directly into the ion source ($250^{o}C$, electron energy 40 eV). The MS was operated in the electron impact mode over the mass range 33-400 a.m.u. with data recorded at a rate of 1 scan s^{-1}.

The volatile products of the heated reaction mixtures described in Chapter 3 were quantified using an automated procedure, developed on the INCOS data system. Libraries of spectra of selected reaction products were prepared by extracting spectral data from appropriate GC-MS analyses. Each analysis was then reverse-searched against each library entry over a restricted range of relative retention times (relative to the internal standard, 1,2-dichlorobenzene). When a successful match was found, the compound was quantified using one characteristic ion, chosen to

minimise interference from adjacent compounds. The ions used for quantitation are listed in Appendix I; for the heterocyclic compounds the quantitation ion was usually the molecular ion. The quantitation lists derived from this procedure were altered manually to correct any errors generated by the automatic procedure, for example due to closely eluting isomers.

The ion areas obtained were assessed in two ways:

(i) The unavailability of authentic samples for many of the heterocyclic compounds made it impossible to measure the absolute quantities of every component; therefore, the results were calculated in terms of relative peak areas. A value for the peak area (total ion area) was calculated as the dividend of the area of the quantitated ion and its relative abundance in the mass spectrum. To take account of variations between replicates these areas were expressed relative to the area given by 1ng 1,2-dichlorobenzene to give relative peak areas (RPA). The RPA represents the contribution made by that compound to the total ion chromatogram. The calculation of relative peak areas is derived algebraically in Section 6.4.2.1.

The total ion area for all the peaks of a chromatogram was obtained using automatic quantitation procedures; this was also expressed relative to the internal standard and termed the 'total RPA'.

(ii) Authentic samples were available for most of the aliphatic compounds examined in Section 3.3. Therefore, it was possible to calculate the actual mass (ng) of each compound collected. A known amount of the compound was applied to a Tenax trap, together with internal standard, and a relative response factor for each compound obtained, enabling the calculation of the absolute quantities collected from the heated reaction mixtures. This procedure is described algebraically in Section 6.4.2.2.

6.4.2.1 Calculation of relative peak areas (RPA)

For a given compound:

A_i = area of quantitation ion
H_i = height of quantitation ion
A_t = total ion area for compound
H_t = total ion height for compound

R = proportion of total ions in mass spectrum contributed by quantitation ion

$$R = \frac{H_i}{H_t} = \frac{A_i}{A_t}$$

$$\therefore \text{ total ion area, } A_t = \frac{A_i}{R} \qquad (1)$$

For the internal standard (1,2-dichlorobenzene):

$$^sR = \frac{^sH_i}{^sH_t} = \frac{^sA_i}{^sA_t}$$

$$\therefore \quad ^sA_t = \frac{^sA_i}{^sR} \qquad (2)$$

The relative peak area (RPA) is the total ion area for the compound expressed as a percentage of the total ion area given by 1ng 1,2-dichlorobenzene:

$$RPA = \frac{At}{^sA_t} \times {}^sm \times 100 \qquad (3)$$

where ^sm = mass of 1,2-dichlorobenzene used as internal standard

Substituting equations 1 and 2 in 3

$$RPA = \frac{A_i}{R} \times \frac{^sR}{^sA_i} \times {}^sm \times 100 \qquad (4)$$

where: ^sm = 65ng

R = a different value for each compound, calculated as the mean value obtained for H_i/H_t from at least three GC-MS runs

^{s}R = 0.259 for m/z 146, the quantitation ion for 1,2-dichlorobenzene

Thus, the RPA could be calculated from the values for A_i and $^{s}A_i$ obtained for each individual GC-MS run.

6.4.2.2 Calculation of quantities (ng) of individual compounds collected

Where the authentic material was available it was possible to compare the signal given by a known amount of the compound with that from the internal standard, and thus to obtain a relative response factor for the compound:

The response factor RF for any compound is given by:

$$RF = \frac{A_i}{m} \qquad (5)$$

where m = the mass of compound present

Thus, for the internal standard:

$$^{s}RF = \frac{^{s}A_i}{^{s}m} \qquad (6)$$

The relative response factor, RRF, can be obtained from:

$$RRF = \frac{RF}{^{s}RF} \qquad (7)$$

The same equations apply to the GC-MS runs of the reaction products, containing unknown quantities of the compounds under study, but a known amount of internal standard (65ng 1,2-dichlorobenzene)

Substituting equations 5 and 6 in 7:

$$RRF = \frac{A_i}{m} \times \frac{^{s}m}{^{s}A_i}$$

$$\therefore \quad m = \frac{A_i}{{}^sA_i} \times \frac{{}^sm}{RRF} \qquad (8)$$

Thus, the quantity, m (ng), of a compound collected could be calculated from the ion areas of the compound and the internal standard.

6.4.3 Gas chromatography-high resolution mass spectrometry

The volatiles from the heated reaction mixtures from the studies described in Chapter 2 were analysed on a Carlo Erba 5300 gas chromatograph coupled to a Kratos MS80 high resolution MS. Desorption and chromatographic conditions were unchanged (except that the front of the column was cooled with a spray of liquid nitrogen). The MS was set at a resolution of 7500 and was scanned from 400-30 a.m.u. Perfluorokerosene was used for calibration and a small amount was continuously bled into the MS source during analysis. The accurate masses obtained for molecular and fragment weights of compounds enabled the molecular (and fragment) formulae to be deduced (Beynon and Williams 1963).

6.4.4 Gas chromatography-infra red spectroscopy

Vapour-phase Fourier transform infrared spectroscopy was performed using a Hewlett Packard (HP) 5890 gas chromatograph fitted with an HP 5965A infrared detector and an HP IRD Chem Station. The effluent from the capillary column passed directly into the light pipe (120mm x 1mm ID) via a heated transfer line (250°C) and from there to a flame ionization detector (FID), fitted in series with the IR detector. The optical systems of the IR detector included an IR source, emitting IR radiation over the range 4000-500 cm^{-1}, and an interferometer, to generate an interference pattern suitable for Fourier transform. The resulting IR beam was passed through the light pipe and the attenuated signal detected by a mercury cadmium telluride detector, sensitive over the range 4000-750cm^{-1}. The detector was operated at an optical resolution of 8 cm^{-1}.

Infra red spectra were recorded during chosen portions of the chromatogram; background IR spectra were obtained by recording suitable regions of baseline which were subtracted from the spectra from peaks of interest.

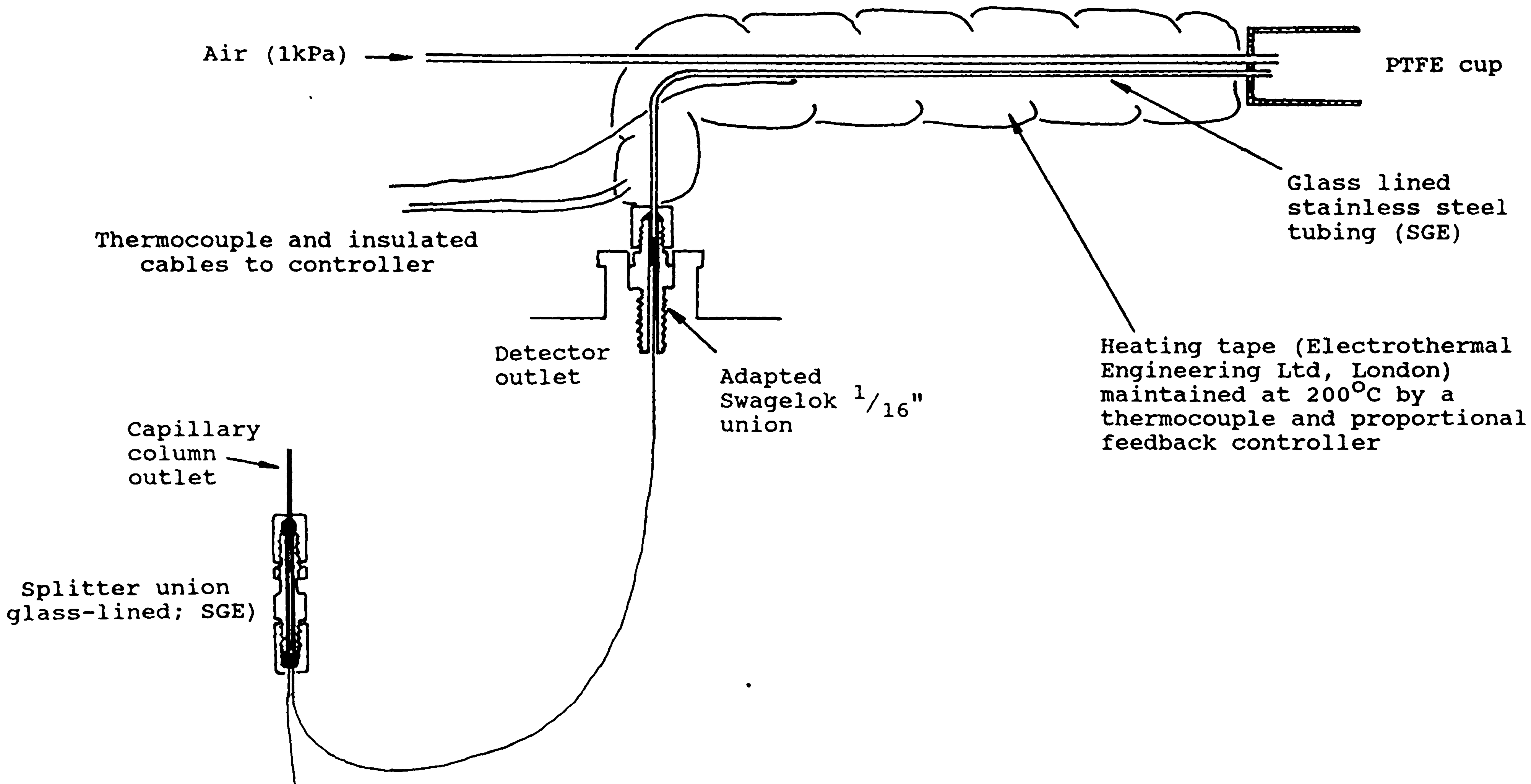
The GC-IR was a factor of approx, 10-1000 times less sensitive than GC-MS, depending on the coefficient of absorption of a given compound. There was a wide variation in coefficients of absorption between different compounds; those of compounds containing carbonyl groups were generally high while unsaturated compounds containing sulphur as the only heteroatom gave very low responses. For example, the alkylfurfurals identified in Section 2.2 gave very clear IR spectra, the thienothiophenes detected in Section 2.3 gave little or no IR data; indeed, it proved necessary to inject 1000 ng of a thienothiophene to obtain a reference IR spectrum.

This variability in sensitivity meant that the GC-IR chromatogram bore little resemblance to the GC-MS total ion trace, derived from analysis of the same sample on a matched column. Alignment of the two sets of data was accomplished by calculation of LRI values and by comparison of both sets of data with the GC-FID trace obtained on the GC-IR instrument; the retention times were the same as those recorded for the IR data while the chromatogram was very similar to that obtained by GC-MS.

6.4.5 Gas chromatography-odour assessment

Gas chromatography (GC) was performed using a Carlo Erba 4200 gas chromatograph fitted with a WCOT fused silica capillary column (50m x 0.32mm ID) coated with CPWAX57CB (Chrompak Ltd). The effluent was split between a flame ionization detector and an "odour port", comprising a PTFE cup (18mm i.d. x 30mm deep) mounted on the side of the GC. A heated (250°C) glass-lined stainless steel tube (1/16" OD) carried the effluent from the split at the end of the column to the "odour port", where it was flushed out by an auxillary air supply (Fig. 6.4C). The aroma of the effluent was assessed by four individuals who marked the chromatogram and noted a description for each aroma detected.

Figure 6.4C: Construction of odour port used for gas chromatography-odour assessment



6.5 ASSESSMENT OF OVERALL AROMA

Heated reaction mixtures, prepared as described in Chapter 2, were diluted either from 2 ml to 10 ml (glycine containing systems) or from 1 ml to 10 ml (where cysteine was the amino acid). These solutions were presented in amber bottles to eight assessors who were asked to generate descriptors for the odour.

The reaction mixtures obtained in Chapter 3 were subjected to a rudimentary evaluation of odour, immediately prior to headspace collection; as each sample was opened, the ampoule was covered in foil to disguise its identity and the odour assessed by three chemists experienced in the use of descriptive terms. Their comments were noted for each of the quadruplicate sets of heated reaction mixtures.

CHAPTER 7

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7. REFERENCES

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APPENDICES

APPENDICES

APPENDIX I

Appendix I lists supplementary information relevant to the identification or quantitation of the aroma compounds described in this Thesis.

Whenever possible, the identification of compounds (Chapter 2) was achieved by comparison with authentic compounds. The sources and LRI values for these compounds are listed in Appendix I. In Chapter 3, quantitation of selected compounds entailed the determination of the area of a characteristic fragment ion for each compound. For the aliphatic compounds (Sec. 3.3), where the authentic substance was usually available, the mass (ng) of each compound collected was calculated using relative response factors (see Sec. 6.4.2). The quantitation ions used and, where appropriate, the relative response factor are listed in Appendix I.

APPENDIX II

Appendix II shows typical gas chromatograms of headspace volatiles from the heated reaction mixtures discussed in Chapters 2 and 3.

Chromatograms obtained by GC-odour assessment and GC-MS of the volatiles from the reactions between glycine and ribose, with and without phospholipid (Sec. 2.2), are shown in Fiures II-A,B,C and D. Figures II-A and B are enlarged versions of Figures 2.2A and B, and are included to allow easier reading of the aroma descriptions. Figures II-E,F,G and H show the chromatograms for the corresponding reaction mixtures containing cysteine (Sec. 2.3).

Figures II-I to Q illustrate typical GC-MS ion chromatograms for the headspace volatiles collected from heated reaction mixtures containing cysteine and ribose, each of four lipids, and also cysteine + ribose heated with each lipid, as described in Chapter 3.

APPENDIX I

Compounds used for the identification and/or quantitation of volatiles from model systems: quantitation ion (QI), relative response factor (RRF), LRI values and source of compound when available.

Compound	Number in Tables:[a] 2.2a	2.3a	3.2c	3.3a,b	MW	QI[b]	RRF[c]	LRI[d] 57CB	52CB	Source[e]
SULPHUR-CONTAINING COMPOUNDS										
Alkylthiophenes and related compounds										
2-Methylthiophene	-	-	1	-	98	98	-	1089	1068	OXF
4,5-Dihydro-2-methylthiophene	-	-	2	-	100	100	-	-	-	n/a
2-Ethylthiophene	-	-	3	-	112	112	-	1161	1151	OXF
2,5-Dimethylthiophene	-	8	4	-	112	112	-	1153	1138	OXF
2,3-Dimethylthiophene	-	12	5	-	112	112	-	1207	1193	*
2-Ethyl-5-methylthiophene	-	17	-	-	126	-	-	1233	1223	OXF
2-Propylthiophene	-	19	-	-	126	-	-	1239	-	OXF
2-Butylthiophene	-	41	6	-	140	140	-	1339	1337	OXF
2-Pentylthiophene	-	57	7	-	154	154	-	1440	1441	OXF
2-Hexylthiophene	-	77	8	-	168	168	-	1545	1547	OXF
2-Heptylthiophene	-	99	-	-	182	-	-	1653	1649	OXF
2-Pentylthiapyran	-	100	9	-	168	168	-	-	-	n/a
cis/trans-2-(1-Hexenyl)-thiophene	-	110	10	-	166	166	-	-	-	n/a
cis/trans-2-(1-Hexenyl)-thiophene	-	114	11	-	166	166	-	-	-	n/a
Acylthiophenes										
2-Formylthiophene	-	109	12	-	112	112	-	1688	1647	ALD
3-Acetylthiophene	-	120	13	-	126	126	-	1772	1733	ALD
2-Acetylthiophene	-	121	14	-	126	126	-	1777	1740	OXF
2-Propionylthiophene	-	-	15	-	140	140	-	1842	1806	OXF
2-Formyl-3-methylthiophene	-	127	16	-	126	126	-	1813	1733	ALD
2-Formyl-5-methylthiophene	-	122	-	-	126	-	-	1780	1730	LAN
3-Ethyl-2-formylthiophene	-	-	17	-	140	140	-	-	-	n/a
A dimethylformylthiophene	-	-	18	-	140	140	-	-	-	n/a
2-Acetyl-3-methylthiophene	-	119	-	-	140	-	-	1760	1714	ALD
2-Propionylthiophene	-	131	-	-	140	-	-	1842	1806	OXF
A thienylethanal	-	-	19	-	126	126	-	-	-	n/a
Heterocyclic thiols										
2-Furanmethanethiol	-	53	20	-	114	114	-	1421	1409	ALD
2-Methyl-3-furanthiol	-	29	21	-	114	114	-	1295	1282	ALD
2-Thiophenethiol	-	80	22	-	116	116	-	1559		*
3-Thiophenethiol	-	-	23	-	116	116	-	-	-	n/a
2-Methyl-3-thiophenethiol	-	86	24	-	130	130	-	-	-	n/a

Compound	Number in Tables: 2.2a	2.3a	3.2c	3.3a,b	MW	QI	RRF	LRI 57CB	52CB	Source
<u>Thiophenones</u>										
Dihydro-3(2H)-thiophenone	-	79	25	-	102	102	-	-	1519	ALD
Dihydro-2-methyl-3(2H)-thiophenone	-	74	26	-	116	116	-	1526	1491	*
<u>trans</u>-Dihydro-2,(4/5)-dimethyl-3(2H)-thiophenone	-	-	27	-	130	130	-	-	-	n/a
<u>cis</u>-Dihydro-2,(4/5)-dimethyl-3(2H)-thiophenone	-	-	28	-	130	130	-	-	-	n/a
Dihydro-(2/5)-ethyl-3(2H)-thiophenone	-	-	29	-	130	74	-	-	-	n/a
<u>Dithianones and trithianes</u>										
1,2-Dithian-4-one	-	-	30	-	134	134	-	-	-	n/a
3-Methyl-1,2-dithian-4-one[d]	-	-	31	-	148	148	-	-	-	n/a
<u>trans</u>-3,(5/6)-Dimethyl-1,2-dithian-4-one	-	-	32	-	162	162	-	-	-	n/a
3-Methyl-1,2,4-trithiane	-	133	33	-	152	152	-	1862	-	*
<u>cis</u>-3,5-Dimethyl-1,2,4-trithiolane	-	87	-	-	152	-	-	1588	-	*
<u>trans</u>-3,5-Dimethyl-1,2,4-trithiolane	-	90	-	-	152	-	-	1610	-	*
<u>Bicyclic compounds</u>										
2,3-Dihydro-6-methyl-thieno[2,3c]furan	-	115	34	-	140	140	-	1697	-	*
Thieno[2,3b]thiophene	-	134	35	-	140	140	-	1857	-	*
Thieno[3,2b]thiophene	-	136	-	-	140	-	-	1876	1833	*
A methylthienothiophene	-	-	36	-	154	154	-	-	-	n/a
A dihydrothienothiophene	-	-	37	-	142	142	-	-	-	n/a
A methyldihydrothienothiophene	-	-	38	-	156	156	-	-	-	n/a
<u>Thiazoles</u>										
Thiazole	-	22	39	-	85	85	-	1248	1213	ALD
2-Methylthiazole	-	20	40	-	99	99	-	-	-	n/a
2,5-Dimethylthiazole	-	35	-	-	113	-	-	1330	1286	*
4,5-Dimethylthiazole	-	47	41	-	113	113	-	1378	1334	OXF
Trimethylthiazole	-	48	42	-	127	127	-	1385	1341	OXF
5-Ethyl-4-methylthiazole	-	58	-	-	127	-	-	1446	-	*
5-Ethyl-2,4-dimethylthiazole	-	60	-	-	141	-	-	1455	-	*
2-Acetylthiazole	-	97	43	-	127	127	-	1639	1604	PYR
<u>Mercaptocarbonyls</u>										
2-mercapto-3-butanone	-	-	44	-	104	104	-	-	-	n/a
2-mercapto-3-pentanone	-	-	45	-	118	57	-	-	-	n/a
3-mercapto-2-pentanone	-	-	46	-	118	75	-	-	-	n/a
1-Mercapto-3-pentanone	-	71	47	-	118	118	-	-	-	n/a
4-Mercapto-4-methyl-2-pentanone	-	46	-	-	132	-	-	1376	-	OXF
<u>Alkanethiols</u>										
1-Heptanethiol	-	18	48	-	132	132	-	-	1230	ALD
1-Octanethiol	-	40	49	-	146	146	-	-	1335	ALD

Compound	Number in Tables: 2.2a	2.3a	3.2c	3.3a,b	MW	QI	RRF	LRI 57CB	52CB	Source
COMPOUNDS WITHOUT SULPHUR										
<u>2-Alkylfurans</u>										
2-Butylfuran	6	5	-	35	124	124	0.275	1122	1119	OXF
2-Pentylfuran	16	15	-	36	138	138	0.259	1219	1216	OXF
2-Hexylfuran	27	33	-	37	152	152	0.265	1316	1315	OXF
2-Heptylfuran	42	54	-	38	166	166	0.228	1416	1418	OXF
2-Octylfuran	66	73	-	39	180	180	0.194	1519	1521	OXF
<u>AcylFurans</u>										
2-furfural	47	61	50	-	96	96	-	1455	1428	FLU
1-(2-furyl)-2-propanone	63	-	51	-	124	124	-	-	-	n/a
2-Acetylfuran	60	70	-	-	110	-	-	1499	-	ALD
2-Acetyl-5-methylfuran	-	91	-	-	124	-	-	1621	-	OXF
2-Propionylfuran	72	82	-	-	124	-	-	1575	-	OXF
<u>Other furans</u>										
Dihydro-2-methyl-3(2H)-furanone	-	25	-	-	100	-	-	1256	-	OXF
2-Furanmethanol	91	-	-	-	98	-	-	1665	-	BDH
Benzofuran	55	-	-	-	118	-	-	1489	-	ALD
1-(2-Furfuryl)-pyrrole	109	128	-	-	147	-	-	1821	-	ALD
<u>Pyrroles</u>										
2-Formyl-1-methylpyrrole	82	-	-	-	109	-	-	1622	-	ALD
2-Acetyl-1-methylpyrrole	88	-	-	-	123	-	-	1660	-	ALD
<u>Pyridines</u>										
Pyridine	14	-	-	-	79	-	-	1215	-	ALD
2-Methylpyridine	18	-	-	-	93	-	-	1237	1179	*
4-Methylpyridine	30	-	-	-	93	-	-	1322	1255	*
2-Pentylpyridine	75	85	52	-	149	93	-	1581	1540	OXF
2-Acetylpyridine	77	-	-	-	121	-	-	1596	-	LAN
<u>Pyrazines</u>										
Methylpyrazine	21	26	53	-	94	94	-	1272	1229	ALD
Ethylpyrazine	32	38	54	-	108	107	-	1339	1306	ALD
2,3-Dimethylpyrazine	34	-	55	-	108	108	-	1354	1306	OXF
2,5-Dimethylpyrazine	31	37	-	-	108	-	-	1331	1283	OXF
2,6-Dimethylpyrazine	33	39	-	-	108	-	-	1336	1290	OXF
Trimethylpyrazine	41	-	57	-	122	122	-	1412	1365	OXF
2-Ethyl-3-methylpyrazine	40	52	-	-	122	-	-	1407	1366	OXF
2-Ethyl-5-methylpyrazine	39	50	56	-	122	122	-	1393	1354	PYR
2-Ethyl-6-methylpyrazine	38	-	-	-	122	-	-	1387	1349	PYR
2-Ethyl-3,5-dimethylpyrazine	51	64	-	-	136	-	-	1466	1425	OXF
2-Ethyl-3,6-dimethylpyrazine	46	-	-	-	136	-	-	1449	1409	OXF
2,5-Diethylpyrazine	50	63	-	-	136	-	-	1463	-	*
Tetramethylpyrazine	54	-	-	-	136	-	-	1484	1436	OXF
2-(1-propenyl)-pyrazine	68	-	-	-		-	-	1552	-	*[f]
<u>Oxazoles</u>										
5-Ethyl-4-methyloxazole	-	-	58	-	111	111	-	-	-	n/a
4-Ethyl-5-methyloxazole	-	-	59	-	111	111	-	-	-	n/a
Trimethyloxazole	15	14	60	-	111	111	-	1213	1173	OXF
4-Ethyl-2,5-dimethyloxazole	-	24	-	-	125	-	-	-	1218	OXF

Compound	Number in Tables: 2.2a	2.3a	3.2c	3.3a,b	MW	QI	RRF	LRI 57CB	LRI 52CB	Source
ALIPHATIC COMPOUNDS										
Hydrocarbons										
Decane	1	1	-	-	142	-	-	1000	1000	KLT
Undecane	5	4	-	-	156	-	-	1100	1100	KLT
Dodecane	13	11	-	-	170	-	-	1200	1200	KLT
Tridecane	25	30	-	-	184	-	-	1300	1300	KLT
Aldehydes										
Hexanal	4	3	-	1	100	82	0.033	1089	1060	POL
Heptanal	11	10	-	2	114	96	0.014	1182	1165	POL
Octanal	22	-	-	3	128	84	0.121	1284	1269	POL
Nonanal	37	49	-	4	142	98	0.113	1384	1375	POL
Decanal	-	-	-	5	156	112	0.057	1493	1480	POL
Hexadecanal	-	-	-	6	240	82	-	-	-	POL
2-Pentenal	-	-	-	7	84	84	0.110	1134	1106	OXF
2-Hexenal	-	-	-	8	98	83	0.201	1220	1197	OXF
2-Heptenal	29	34	-	9	112	83	0.164	1323	1296	OXF
2-Octenal	43	-	-	10	126	83	0.179	1426	1402	OXF
2-Nonenal	67	-	-	11	140	83	0.077	1532	1508	OXF
2-Decenal	-	-	-	12	154	83	0.087	1643	1615	OXF
2-Undecenal	100	-	-	13	168	83	0.163	1753	1723	OXF
2,4-Heptadienal	56	-	-	14	110	81	0.755	1493	1460	ALD
2,4-Octadienal	-	-	-	15	124	81	0.8[g]	-	-	n/a
2,4-Nonadienal	-	-	-	16	138	81	0.832	1705	1668	ALD
trans,cis/cis,trans 2,4-Decadienal	-	-	-	17	152	81	0.7[g]	-	-	ALD[f]
tr,tr-2,4-Decadienal	108	126	-	18	152	81	0.691	1816	1772	ALD
2,4-Undecadienal	-	-	-	19	166	81	0.7[g]	-	-	n/a
Ketones										
2-Hexanone	-	-	-	20	100	100	0.103	1092	1063	KLT
2-Heptanone	12	9	-	21	114	71	0.078	1182	1167	KLT
2-Octanone	-	27	-	22	128	71	0.235	1282	1267	KLT
2-Nonanone	-	-	-	23	142	71	0.322	1384	1371	KLT
2-Decanone	-	68	-	24	156	71	0.377	1489	1478	KLT
2-Undecanone	76	-	-	-	170	-	-	1598	-	KLT
2-Tridecanone	107	-	-	-	198	-	-	1811	-	KLT
3-Octanone	19	21	-	25	128	99	0.368	1253	1238	ALD
4-Methyl-2-pentanone	-	-	-	26	100	100	0.034	1012	-	ALD
4-Methyl-3-penten-2-one	9	6	-	27	98	98	0.316	1139	1113	ALD
6-Methyl-5-hepten-2-one	?	36	-	-	126	-	-		1315	FLU
1-Octen-3-one	24	-	-	-	126	-	-	1399	-	OXF
3-Nonen-2-one	61	-	-	-	140	-	-	1513	-	LAN
2,3-Pentanedione	3	-	-	-	100	-	-	1053	-	ALD
2,3-Hexanedione	7	-	-	-	114	-	-	1125	-	OXF
3,4-Hexanedione	8	-	-	-	114	-	-	1133	-	OXF
1-Hydroxy-2-propanone	26	-	-	-	74	-	-	1308	-	ALD
3-Hydroxy-2-butanone	23	-	-	-	88	-	-	1286	-	ALD

Compound	Number in Tables: 2.2a	2.3a	3.2c	3.3a,b	MW	QI	RRF	LRI 57CB	52CB	Source
Alcohols										
1-Pentanol	20	23	-	-	88	-	-	1253	1219	BDH
1-Hexanol	35	44	-	28	102	69	0.249	1357	1321	FLU
1-Heptanol	49	62	-	29	116	70	0.587	1460	1424	FLU
1-Octanol	71	81	-	30	130	70	0.374	1562	1525	FLU
1-Nonanol	89	102	-	31	144	70	0.400	1667	1627	KLT
1-Decanol	104	-	-	-	158	-	-	1770	-	FLU
2-Ethyl-1-hexanol	58	69	-	-	130	-	-	1493	1469	ALD
1-Octen-3-ol	-	59	-	32	128	72	0.176			OXF
trans-2-Octen-1-ol	80	92	-	33	128	68	0.165	1451	1429	OXF
cis-2-Octen-1-ol	81	93	-	34	128	68	0.165[g]	-	-	n/a
CYCLIC COMPOUNDS										
Benzaldehyde	62	-	-	-	106	-	-	1518	-	BDH
3,5-Dimethyl-2-cyclohexen-1-one	83	94	-	-	124	-	-	1629	-	ALD
INTERNAL STANDARD										
1,2-dichlorobenzene					146	146	1.000	1472	1455	BDH

a The fact that a compound is not included in a particular table, does not necessarily indicate its absence in the reaction mixture concerned.

b QI = Quantitation ion used for quantitative studies in Sections 3.2 and 3.3

c RRF = Relative response factor, used in Section 3.3:

$$RRF = \frac{\text{ion area (cpd)}}{\text{mass injected (cpd)}} \times \frac{\text{mass injected (internal standard)}}{\text{ion area (internal standard)}}$$

d LRI = Linear retention index, calculated relative to the n-alkanes for capillary columns coated with either CPWAX57CB (57CB) or CPWAX52CB (52CB)

e Gifts of aroma chemicals from the following companies are gratefully acknowledged (indicated by *):

Dalgety Spillers Ltd (Cambridge), Firmenich SA (Geneva, Switzerland) Givaudin AG (Dulsendorf, Switzerland), Haarman & Reimer GmbH (Holzminden, FRG), Long Ashton Research Station (Bristol, UK), PFW (Amersfoort, Netherlands), TNO-CIVO Food Analysis Institute, (Zeist, Netherlands)

Other chemicals were purchased from:

OXF	Oxford Chemicals, Oxford
ALD	Aldrich, Poole
PYR	Pyrazine Specialities, Atlanta, Georgia, USA
FLU	Fluka Chemicals Ltd, Glossop
POL	Polyscience Inc. (from Windsor Laboratories Ltd, Slough)
KLT	Koch-Light Ltd, Haverhill
LAN	Lancaster Synthesis, Morecambe
BDH	BDH Chemicals, Bristol

Compounds marked 'n/a' were not available

f Present as impurities in samples of closely related compounds from these sources (trans,trans-2,4-decadienal and 2-(1-isopropenyl)-pyrazine).

g Estimated from RRF of closely related compound.

APPENDIX II

Typical gas chromatograms of headspace volatiles from the heated reaction mixtures containing glycine or cysteine, ribose and/or various lipids, as discussed in Chapters 2 and 3.

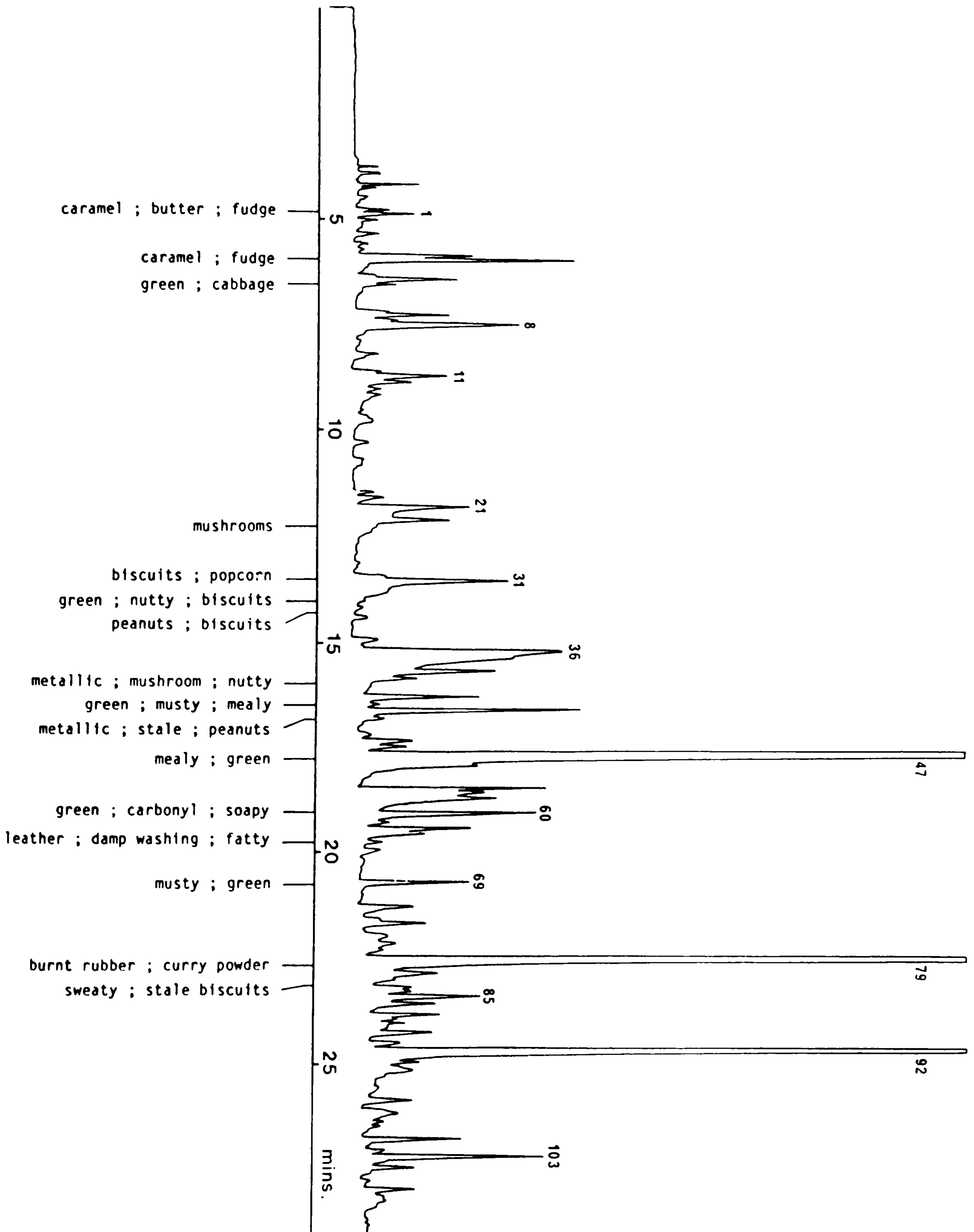

Figure II-A: Typical gas chromatogram of the volatile products of the reaction between glycine and ribose, showing positions of selected compounds (numbers refer to Table 2.2a) and a summary of the aromas detected in the column effluent. FID sensitivity: 64 pA full scale (Sec. 2.2).

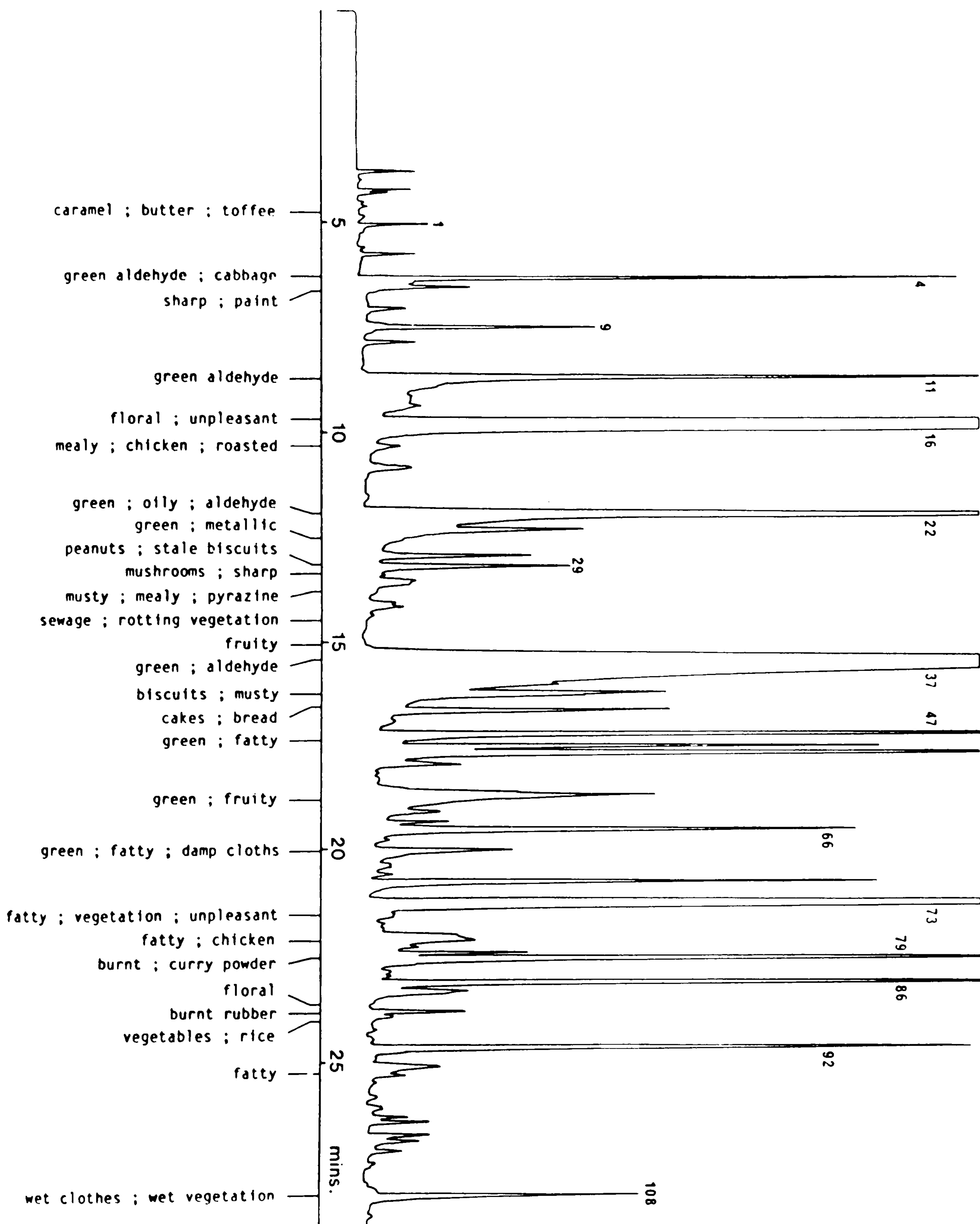

Figure II-B: Typical gas chromatogram of the volatile products of the reaction between glycine, ribose and phospholipid, showing positions of selected compounds (numbers refer to Table 2.2a) and a summary of the aromas detected in the column effluent. FID sensitivity: 256 pA full scale (Sec. 2.2).

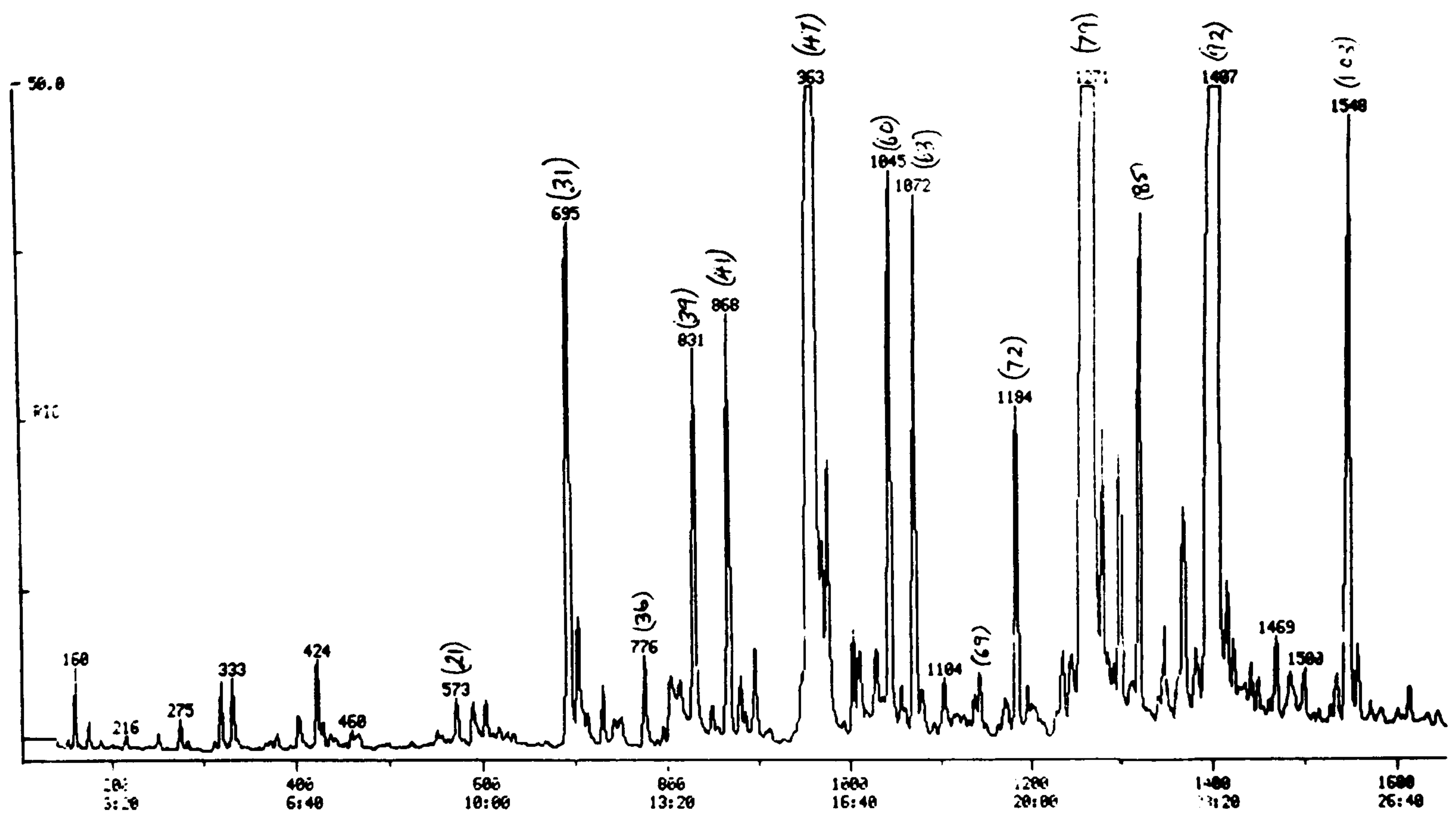

Figure II-C: Typical GC-MS ion chromatogram of the volatile products of the reaction between glycine and ribose, showing positions of selected compounds (numbers refer to Table 2.2a; Sec. 2.2).

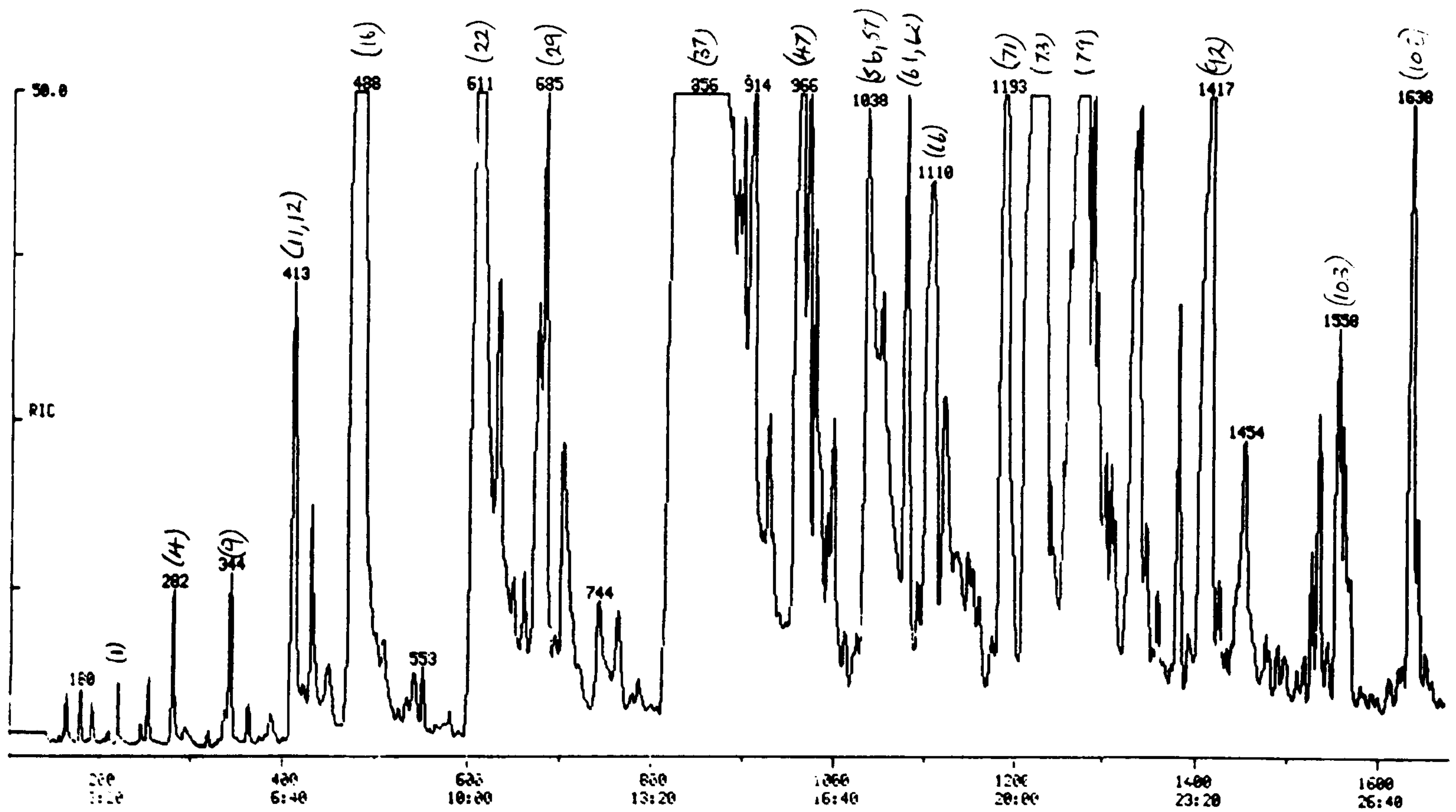

Figure II-D: Typical GC-MS ion chromatogram of the volatile products of the reaction between glycine, ribose and phospholipid, showing positions of selected compounds (numbers refer to Table 2.2a; Sec. 2.2).

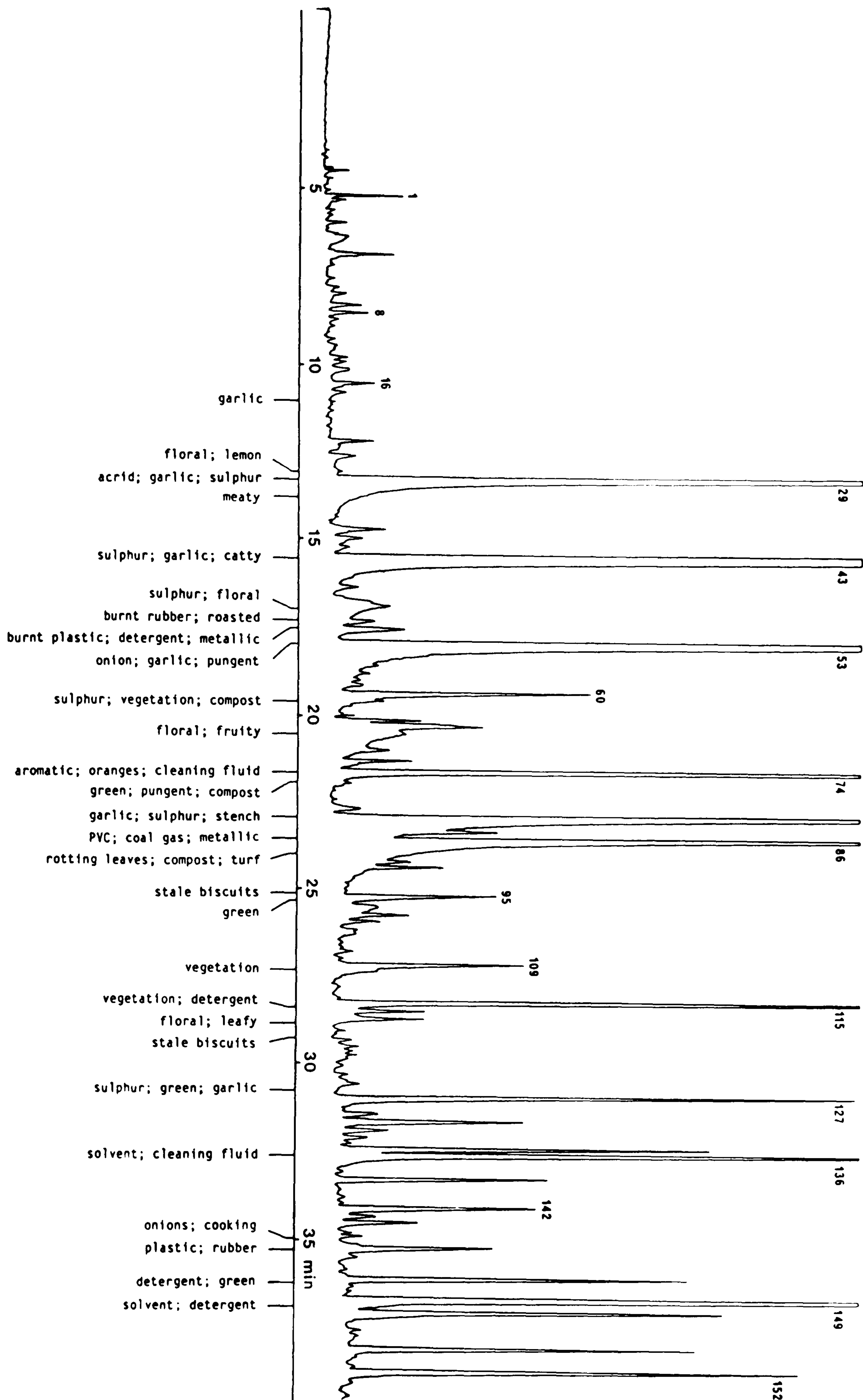

Figure II-E: Typical gas chromatogram of the volatile products of the reaction between cysteine and ribose, showing positions of selected compounds (numbers refer to Table 2.3a) and a summary of the aromas detected in the column effluent. FID sensitivity: 16 pA full

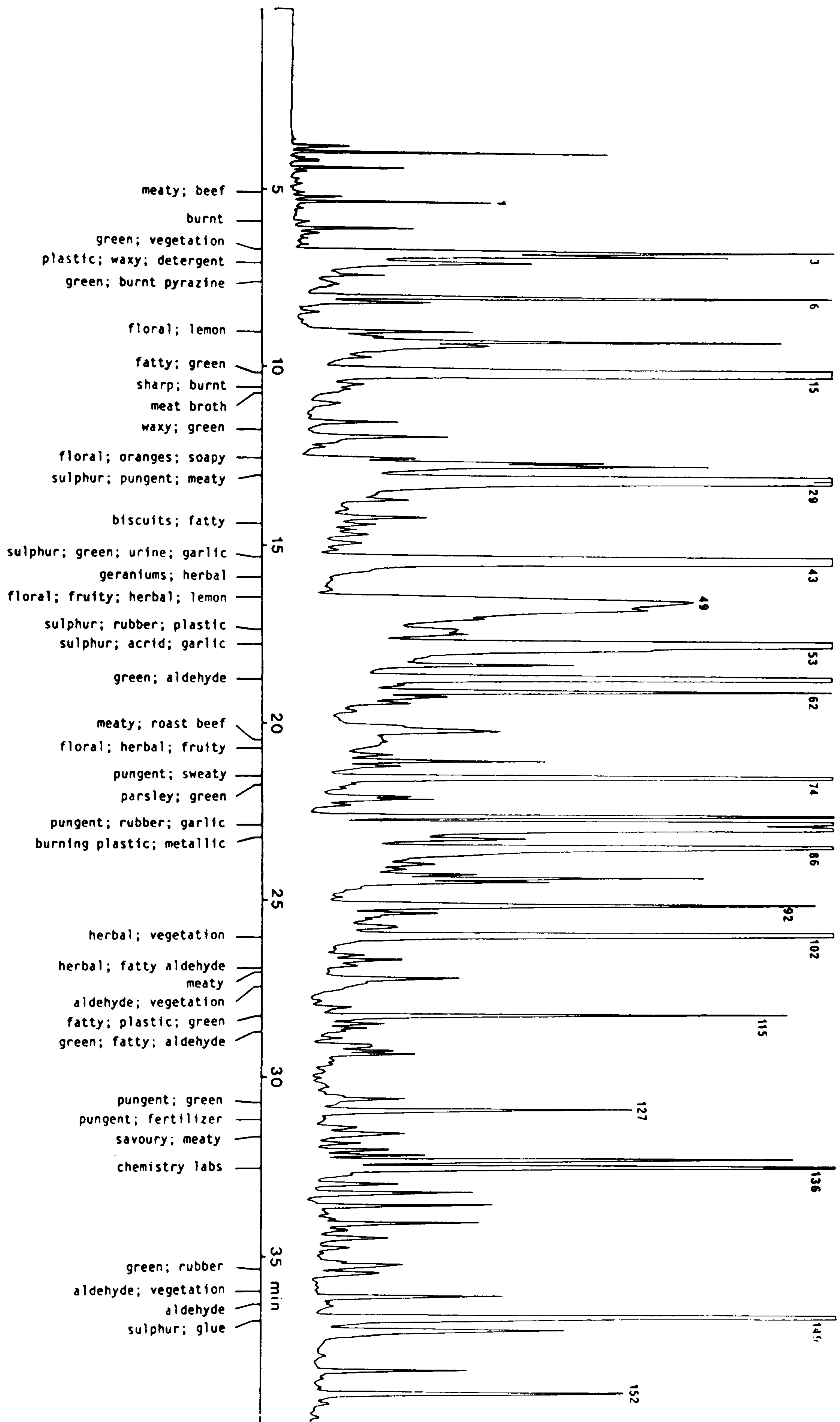

Figure II-F: Typical gas chromatogram of the volatile products of the reaction between cysteine, ribose and phospholipid, showing positions of selected compounds (numbers refer to Table 2.3a) and a summary of the aromas detected in the column effluent. FID sensitivity: 32 pA full scale (Sec. 2.3).

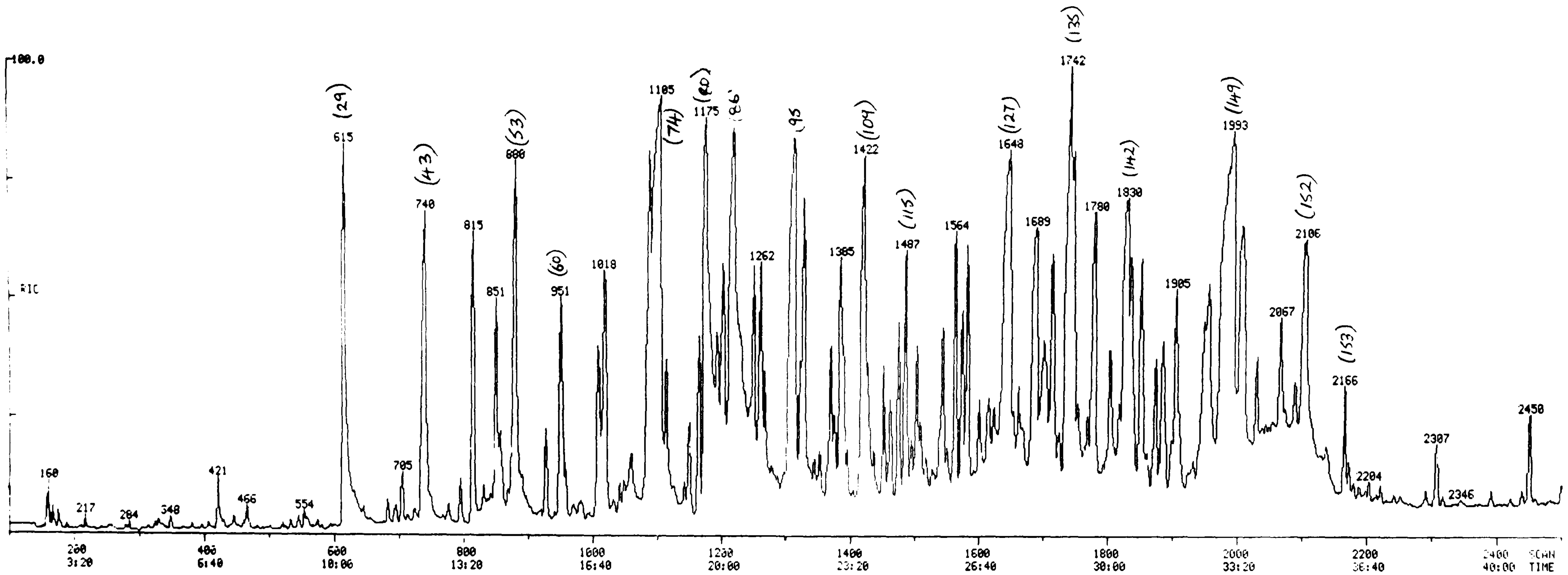

Figure II-G: Typical GC-MS ion chromatogram of the volatile products of the reaction between cysteine and ribose, showing positions of selected compounds (numbers refer to Table 2.3a; Sec. 2.3).

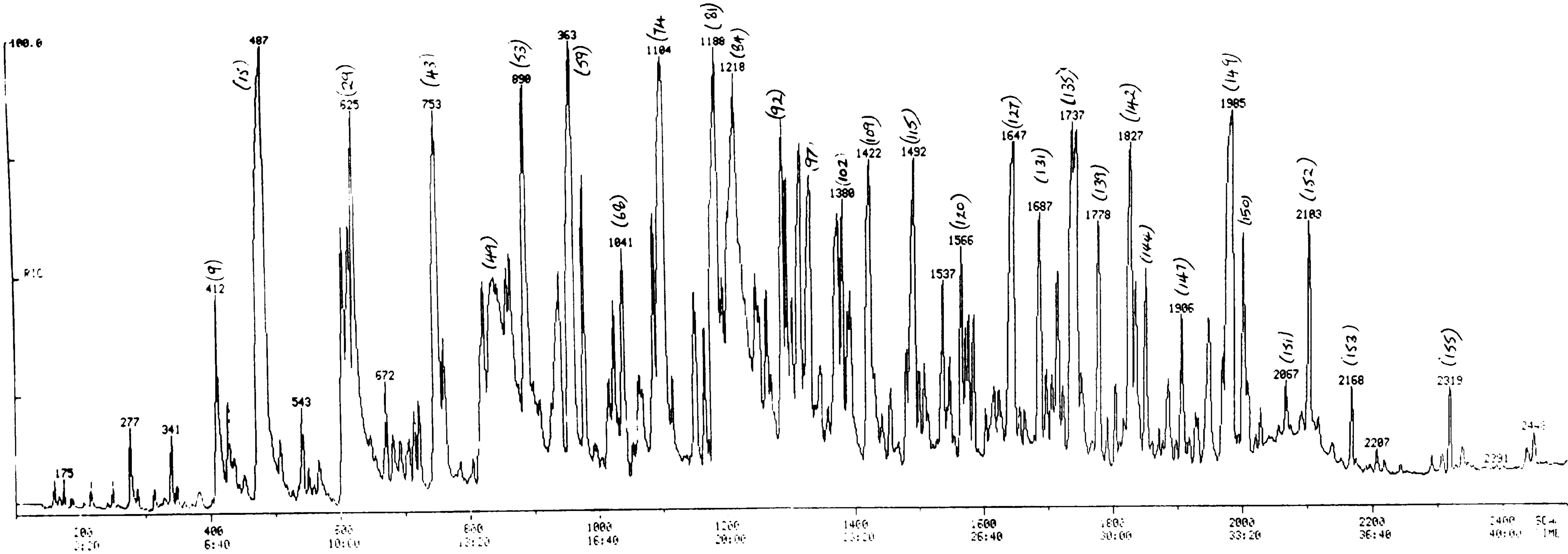

Figure II-H: Typical GC-MS ion chromatogram of the volatile products of the reaction between cysteine, ribose and phospholipid, showing positions of selected compounds (numbers refer to Table 2.3a; Sec. 2.3).

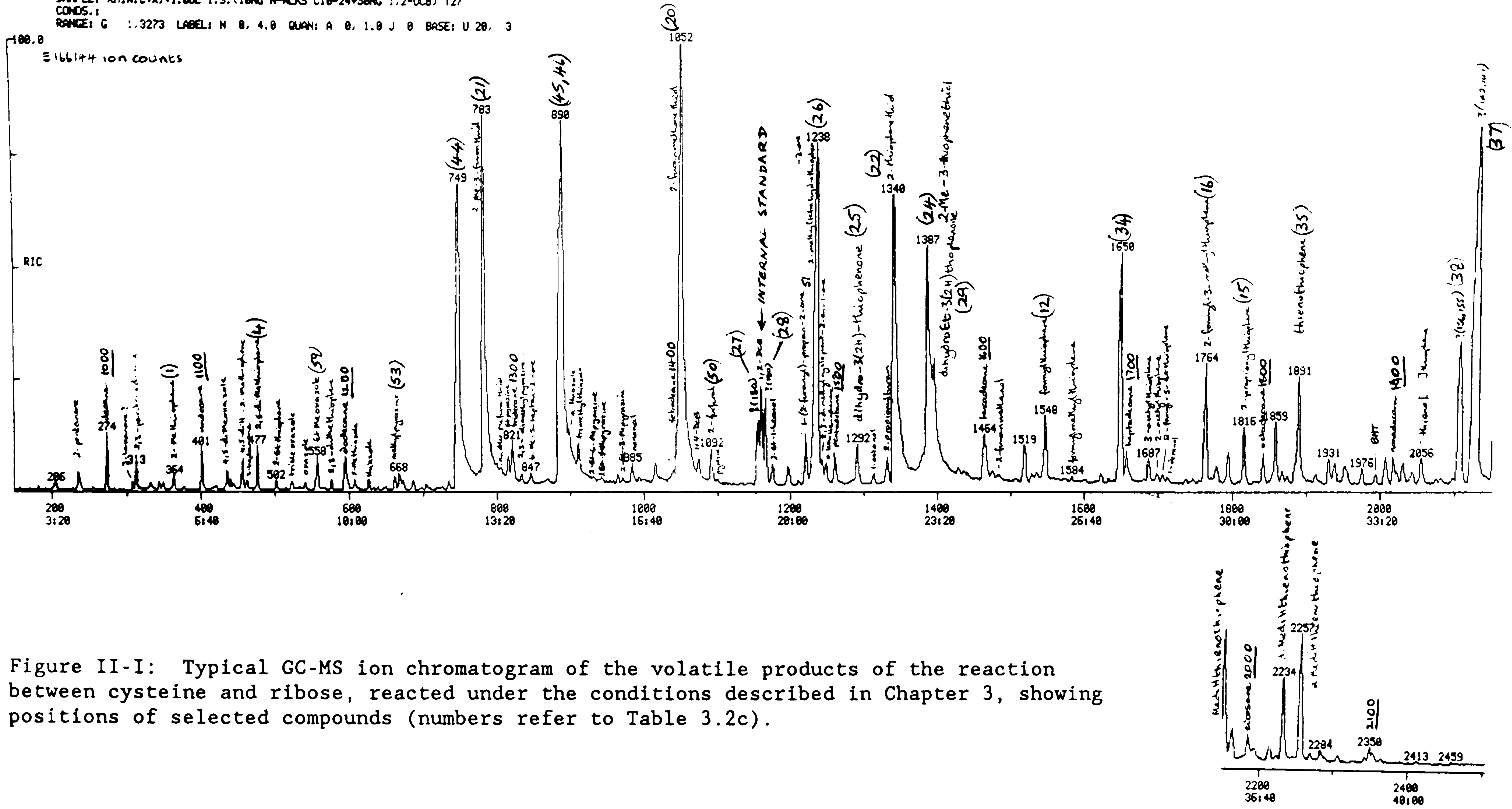

Figure II-I: Typical GC-MS ion chromatogram of the volatile products of the reaction between cysteine and ribose, reacted under the conditions described in Chapter 3, showing positions of selected compounds (numbers refer to Table 3.2c).

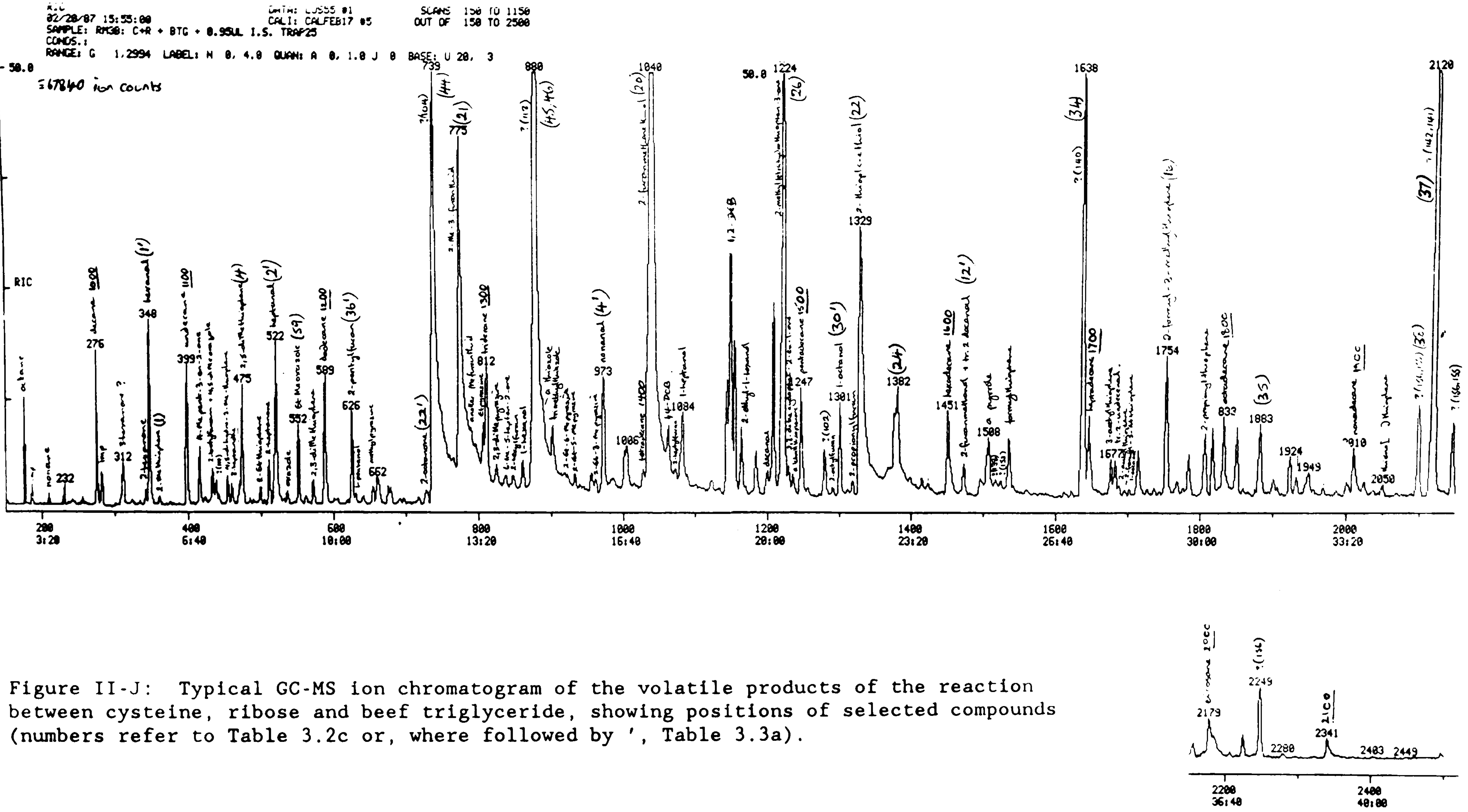

Figure II-J: Typical GC-MS ion chromatogram of the volatile products of the reaction between cysteine, ribose and beef triglyceride, showing positions of selected compounds (numbers refer to Table 3.2c or, where followed by ', Table 3.3a).

RIC
02/24/87 11:29:00
SAMPLE: RMSB:BPL + C+R + 1.0UL I.S. TRAP27
CONDS.:
RANGE: G 1,2940 LABEL: N 0, 4.0 QUAN: A 0, 1.0 J 0 BASE: U 20, 3

DATA: LJS62 #1
CALI: CALFEB17 #5

SCANS 150 TO 1150
OUT OF 150 TO 2500

Figure II-K: Typical GC-MS ion chromatogram of the volatile products of the reaction between cysteine, ribose and beef phospholipid, showing positions of selected compounds (numbers refer to Table 3.2c or, where followed by ', Table 3.3a).

Figure II-L: Typical GC-MS ion chromatogram of the volatile products of the reaction between cysteine, ribose and egg phosphatidylcholine, showing positions of selected compounds (numbers refer to Table 3.2c or, where followed by ', Table 3.3a).

RIC
02/23/87 14:49:00
SAMPLE: RM98: C+R + CEPH + 1.05UL I.S. TRAP24
CONDS.:
RANGE: G 1,3014 LABEL: N 0, 4.0 QUAN: A 0, 1.0 J 0 BASE: U 20, 3
DATA: CJ539 #1
CALI: CALFEB17 #5
SCANS 150 TO 1150
OUT OF 150 TO 2500

Figure II-M: Typical GC-MS ion chromatogram of the volatile products of the reaction between cysteine, ribose and egg phosphatidylethanolamine, showing positions of selected compounds (numbers refer to Table 3.2c or, where followed by ', Table 3.3a).

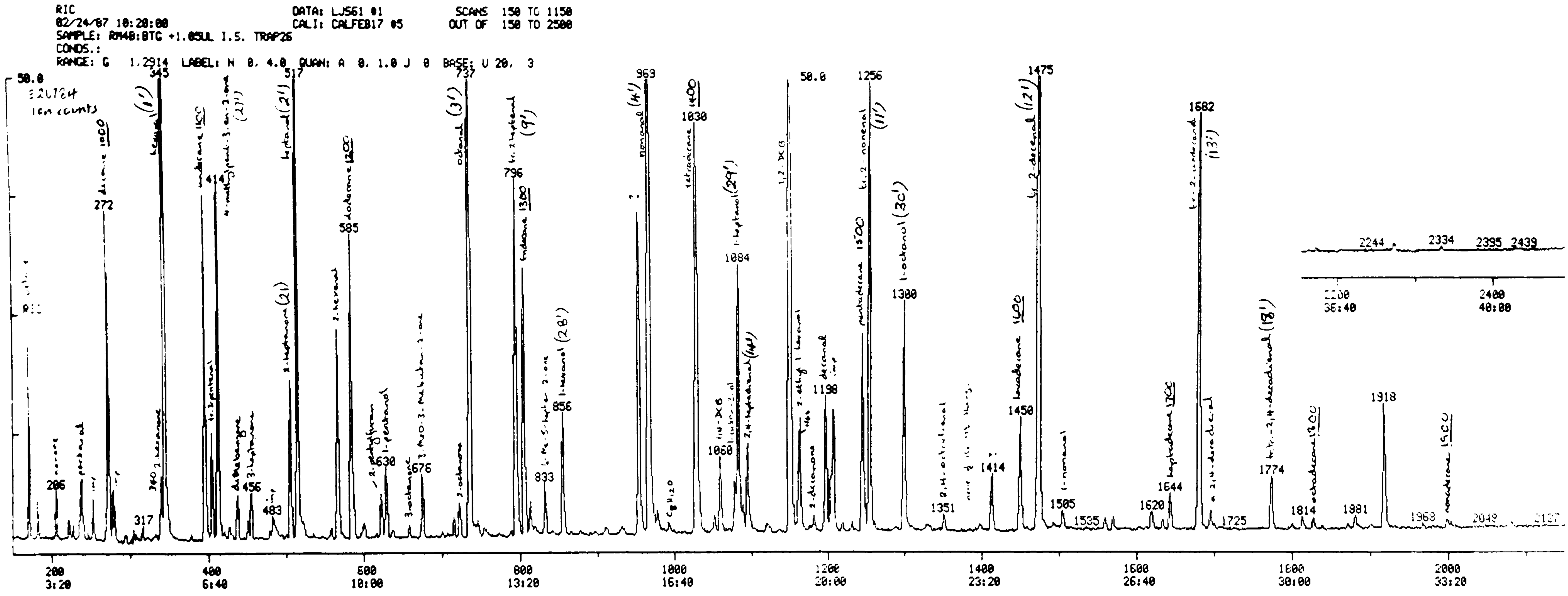

Figure II-N: Typical GC-MS ion chromatogram of the volatile products obtained on heating beef triglyceride, showing positions of selected compounds (numbers refer to Tables 3.3a and b).

Figure II-0: Typical GC-MS ion chromatogram of the volatile products obtained on heating beef phospholipid, showing positions of selected compounds (numbers refer to Table 3.3a and b).

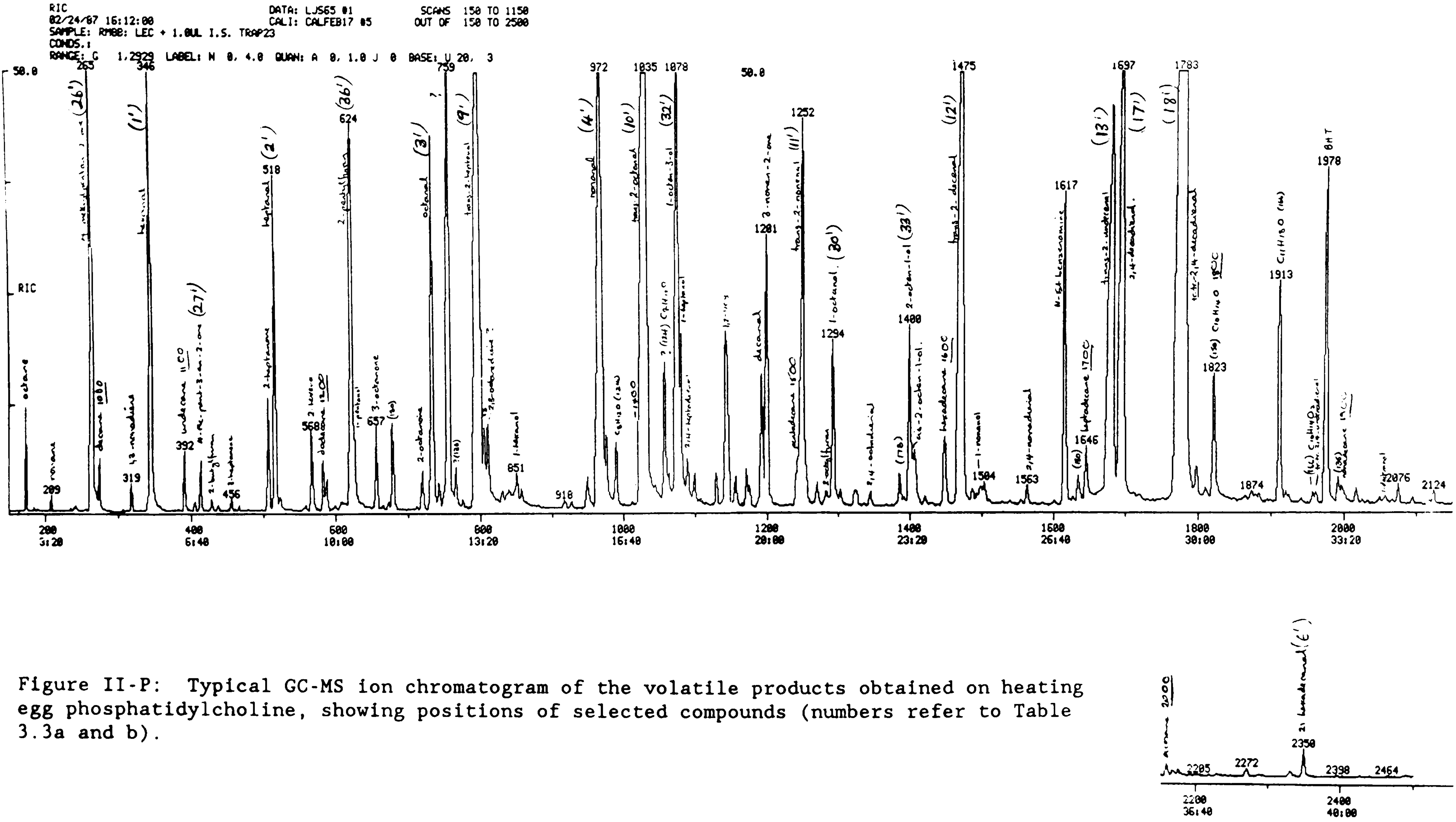

Figure II-P: Typical GC-MS ion chromatogram of the volatile products obtained on heating egg phosphatidylcholine, showing positions of selected compounds (numbers refer to Table 3.3a and b).

Figure II-Q: Typical GC-MS ion chromatogram of the volatile products obtained on heating egg phosphatidylethanolamine, showing positions of selected compounds (numbers refer to Table 3.3a and b

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